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Acute pulmonary embolism: from coagulation to epidemiology

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Academic dissertation

To be presented for public discussion with the permission of the Faculty of Medicine of the University of Helsinki in lecture room 2 Biomedicum, on the 1st of Oktober, at 12 noon

Helsinki 2021

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ISBN 978-951-51-7448-2 (nid.)
ISBN 978-951-51-7449-9 (PDF)

1. Abstract

Pulmonary embolism (PE), together with deep venous thrombosis (DVT), is the main manifestation of venous thromboembolism (VTE). Initial activation of the coagulation system, necessary for the development of VTE, is assumed to occur mainly in the venous valves of the lower extremity veins.

The clinical manifestations of VTE vary widely from an asymptomatic incidentally detected distal DVT to a life-threatening extensive PE, but the factors regulating its extent are known only partially. Also, data regarding the reflection of the dynamic thrombotic process on several plasma markers of haemostasis are scarce.

The aims of this thesis were to evaluate the time-dependent effects of acute PE on plasma levels of haemostatic markers (study I), how VTE extent, PE localization, concomitant DVT and plasma levels of haemostatic markers are related (studies II and III), and the PE mortality trends in Finland during the last 20 years (study IV).

The cohort that was analysed in studies I–III consisted of 63 PE patients and 15 healthy controls.

Laboratory analyses of the plasma levels of haemostatic markers (e.g. D-dimer, factors V (FV), VIII (FVIII) and XIII (FXIII), von Willebrand factor antigen (vWF:Ag), soluble thrombomodulin) were performed in the acute phase shortly after diagnosis and in the stable phase 7 months later. Intraindividual comparisons were made between the two time points, as well as comparisons with healthy controls.

In conclusion, in studies I–III it was seen that acute PE causes changes to analysed haemostatic markers (plasma levels of FXIIIa and vWF:Ag), and significant negative correlation between FV plasma level and platelet count with VTE volume was also seen. Interestingly, preceding antiplatelet therapy was associated with smaller VTE volume. The coexistence of DVT in these PE patients was associated with a more central location of the PE, but the analysed haemostatic markers and patient characteristics were similar between patients with or without coexisting DVT.

In study IV, we analysed the data on PE mortality in Finland during 1996–2017 from the death certificate archive – a registry maintained by Statistics Finland. Overall, PE mortality decreased by almost 28% during follow-up and the decrease was twofold in females compared with males. The impact of the population-level autopsy rate on detected PE mortality was evaluated and a statistically significant association was seen. Interestingly, the annual PE mortality decreased as much in the period when the autopsy rate remained unchanged (1996–2009) as in the period when the autopsy rate started to decline rapidly (2010–2017), but when the

impact of the decrease in the autopsy rate was estimated, the PE mortality plateaued in 2009.

Tiivistelmä

Keuhkoveritulppa ja alaraajojen syvien laskimoiden tukos ovat laskimotukoksen erilaisia ilmentymiä, jotka usein esiintyvät samanaikaisesti. Hyytymisjärjestelmän aktivaatio on välttämätöntä laskimotukoksen syntymiselle ja yleisesti ajatellaan, että tämä aktivaatio käynnistyy yleisimmin alaraajojen laskimoläpissä. Laskimotukoksen ilmiasu voi vaihdella suuresti, hengenvaarallisesta keskeiset keuhkovaltimot tukkivasta tukoksesta pienen etäisen alaraajalaskimon tukokseen, mutta on suurelta osin epäselvää, mitkä tekijät selittävät ilmiasun eroavaisuuksia. Tukoksen kehittymisen aikaiset muutokset plasmasta mitattaviin hyytymisjärjestelmän tekijöihin tunnetaan myös huonosti.

Tämän väitöskirjan tarkoituksena oli tarkastella akuutin keuhkoveritulpan aiheuttamia muutoksia veren hyytymiseen osallistuvien tekijöiden plasmapitoisuuksissa ja keuhkoveritulpan ilmiasun yhteyttä havaittuihin muutoksiin (osatyöt I–III). Lisäksi tutkimme keuhkoveritulpan aiheuttamien kuolemantapausten määrän vaihtelua Suomessa vuosien 1996–2017 aikana (osatyö IV).

Tutkimuksen I–III osatöissä tutkittiin 63:a akuutin keuhkoveritulpan sairastanutta henkilöä ja lisäksi 15:a iän ja sukupuolen mukaan kaltaistettua verrokkia. Verikokeet otettiin kaikilta potilailta heti diagnoosin jälkeen (akuuttivaihe) ja 7 kuukauden päästä (vakaa vaihe). Laboratoriossa tutkittiin seuraavia hyytymisjärjestelmään osallistuvia tekijöitä: D-Dimeeri, Hyytysmistekijät V, VIII ja XIIIa, von Willebrand tekijän antigeeni (vWF:Ag) ja liukoinen thrombomoduliini.

Kokonaisuudessaan osatöissä I–III havaittiin, että akuutti keuhkoveritulppa aiheuttaa plasmasta mitattavia muutoksia hyytymisjärjestelmän tekijöissä FXIIIa ja vWF:Ag. Keuhkoveritulpan ja mahdollisesti sen ohella esiintyvän laskimotukoksen kokonaistilavuus vaihteli suuresti potilaiden välillä ja korreloi negatiivisesti hyytymistekijä V:n ja verihiutaleiden määrän kanssa. Lisäksi havaittiin, että keuhkoveritulppaa edeltänyt verihiutale-estäjien käyttö oli yhteydessä pienempään laskimotukoksen kokonaistilavuuteen. Potilaskohortissamme noin puollella potilaista todettiin keuhkoveritulpan lisäksi alaraajan laskimoiden hyytymä. Kaikilla niillä potilailla, joilla keuhkoveritulppa sijaitsi keskeisissä keuhkovaltimoissa, todettiin myös alaraajalaskimoiden tukos. Yhdelläkään niistä potilaista potilaalla, jolla oli ainoastaan pienen etäisen keuhkolaskimon veritulppa ei todettu alaraajalaskimoiden tukosta. Samanaikaisella alaraajan laskimotukoksella ei ollut yhteyttä plasmasta mitattujen hyytymistekijöiden määrään tai muihin potilaskohtaisiin ominaisuuksiin.

Neljännessä osatyössä raportoimme keuhkoveritulppaan liittyvän kuolleisuuden muutoksia vuosien 1996–2017 aikana. Tiedot keuhkoveritulppaan kuolleista potilaista kerättiin Tilastokeskuksen kuolinsyyrekisteristä. Keuhkoveritulppaan liittyvä kuolleisuus väheni noin 28% seuranta-aikana. Kuolleisuuden väheneminen oli kaksi kertaa suurempaa naisilla kuin miehillä. Ruumiinavausmäärien vaikutusta tilastoituihin keuhkoveritulppan liittyvien kuolemien toteamiseen tarkasteltiin myös ja näiden välillä oli tilastollisesti merkitsevä yhteys. Keuhkoveritulppa kuolleisuuden vuotuinen lasku oli saman suuruista ajanjaksona, jolloin ruumiinavausten määrä pysyi muuttumattomana (1996–2009) tai laski merkittävästi (2010–2017), mutta ruumiinavausten määrän laskun todennäköisen vaikutuksen huomioimisen jälkeen keuhkoveritulppakuolleisuus pysyi muuttumattomana vuoden 2009 jälkeen.

Contents

3. List of original publications

I. Sane M, Granér M, Laukkanen JA, Harjola V-P, Mustonen P. Plasma levels of haemostatic factors in patients with pulmonary embolism on admission and seven months later. Int J Lab Hematol. 2018;40:66–71.

II. Sane M, Granér M, Raade M, Piilonen A, Laukkanen JA, Harjola V-P, Mustonen P. Combined volume of pulmonary embolism and deep venous thrombosis—Association with FV, platelet count, and D-dimer. Int J Lab Hematol. 2018;40(5):e102–e104.

III. Sane MA, Laukkanen JA, Granér MA, Piirilä PL, Harjola VP, Mustonen PE. Pulmonary embolism location is associated with the co-existence of the deep venous thrombosis. Blood Coagul Fibrinolysis. 2019;30(5):188–192.

IV. Sane M, Sund R, Mustonen P. Evaluation of the impact of simultaneous changes in the autopsy rate on mortality trend of pulmonary embolism, Finland, 1996–2017. Submitted.

4. Abbreviations

AAPC	Average annual percentage change
ASA	Acetylsalicylic acid
CRP	C-reactive protein
CT	Computed tomography
CTPA	Computed tomography pulmonary angiography
DVT	Deep venous thrombosis
ELISA	Enzyme-linked immunosorbent assay
ESC	European Society of Cardiology
FENO	Fractional exhaled nitric oxide
Fg	Fibrinogen
FII	Factor II
FV	Factor V
FVII	Factor VII
FVIII	Factor VIII
FIX	Factor IX
FX	Factor X
FXIII	Factor XIII
ICD	International Statistical Classification of Diseases
LMWH	Low-molecular-weight heparin
PE	Pulmonary embolism
PL	Plasma level
TF	Tissue factor
V/Q scan	Ventilation-perfusion lung scintigraphy
VTE	Venous thromboembolism
vWF:Ag	von Willebrand factor antigen

5. Introduction

Pulmonary embolism (PE) is the third most common cause of cardiovascular mortality (Weitz 2011) and it is estimated that PE affects more than 1:1000 subjects yearly. The incidence of PE has more than doubled in the past 20 years in developed countries (Keller et al. 2020, Kempny et al. 2019, Wiener et al. 2011) but the data from Finland have not been published. Despite the increased incidence, PE mortality has decreased in recent decades (Barco et al. 2020 B, Martin et al. 2020, Shiraev et al. 2013, Olié et al. 2015), which may indicate that PE prognosis has improved.

Venous thromboembolism (VTE) is an umbrella term for both PE and deep venous thrombosis (DVT). PE is considered to originate in the vast majority of cases from thrombosis of the lower extremity deep veins (DVT) (Kearon 2003). The valves of the leg veins are considered as the sites for the development of thrombosis as slow blood flow in that area favours the development of hypoxia in the endothelium of venous valves and concomitant activation of the coagulation system (Hamer et al. 1981, Wakefield et al. 2008). The formation and growth of the initial thrombi, which can detach causing PE, are influenced by various genetic and acquired factors (Konstantinides et al. 2020). Studies have shown that in approximately half of the PE patients DVT is not found and in half of the patients with DVT there are no signs of PE (Stein et al. 2010, van Langevelde et al. 2013).

The triggers and events of the coagulation system that participate in the development of thrombosis *in vitro* are well known (Hoffman and Monroe 2001). In general, the stepwise activation of the different coagulation factors leads to the development of a fibrin network to which blood cells can attach (Figure 1). The manifestation of VTE can vary greatly from minimal distal DVT to extensive proximal PE but knowledge about the pathophysiology explaining this variation is limited. When the properties of *in vitro* formed thrombus in patients with different VTE manifestations have been studied, different properties of the fibrin network affecting the tendency to embolize were found (Undas et al. 2009, Martinez et al. 2014). Similar findings were also seen when *in vivo* formed thrombi of DVT and PE patients were evaluated (Chernysh et al. 2020). When compared with a purified *in vitro* environment, the *in vivo* coagulation process is much more complex due to the interference of, for example, endothelial pro- and anticoagulative properties, localization of coagulation factor complexes on the cellular surfaces, and cellular release and uptake of factors and regulatory compounds (Hoffman and Monroe 2001, Wakefield et al. 2008) (Figure 1). The circulating plasma levels (PLs) of factors that participate in the coagulation process can be measured (Fareed et al. 1998). A

few studies have evaluated the PLs of haemostatic markers in patients with acute VTE but their progression during follow-up and the association of these markers with the VTE manifestation have been less studied (Yamada et al. 1995, Kucher et al. 2003, Tichelaar et al. 2012, Tang et al. 2017).

The aims of this thesis were to assess 1) how PLs of haemostatic markers (D-dimer, fibrinogen (Fg), factors V (FV), VIII (FVIII) and XIIIa (FXIIIa), von Willebrand factor antigen (vWF:Ag) and soluble thrombomodulin are affected by acute PE, 2) how PLs of haemostatic markers D-dimer, FV, FVIII and FXIIIa, vWF:Ag, soluble thrombomodulin, platelet count and red blood cell count are associated with the extent of VTE, 3) whether PE patients with or without coexisting DVT differ and finally 4) how the PE mortality trend has changed in Finland in the past 20 years.

6. Review of the literature

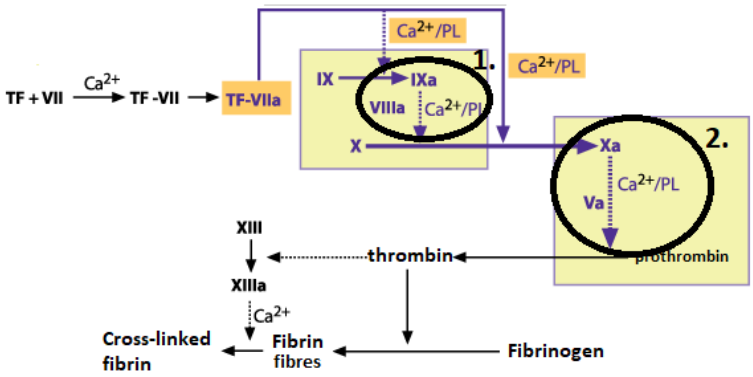
6.1 Coagulation system and pulmonary embolism pathophysiology

6.1.1 Blood coagulation and clot formation

Blood coagulation is a normal response to injury and a general view of this system is presented. The key factors in the coagulation system are a series of proteins – coagulation factors – represented by Roman numerals (Figure 1). Synthesis of these factors occurs mainly in the liver and they circulate as inactive zymogens (Dashty et al. 2012). Coagulation factors function in a complicated interplay in which one activates another, forming complexes on the phospholipid surfaces of platelets and in a cascade-like fashion end up producing fibrin, which forms the structure where blood cells can adhere to cover the injury site (Hoffman and Monroe 2001).

An overview of the coagulation system is summarized by the current cell-based view presented by Hoffman and Monroe (2001). The coagulation cascade includes three overlapping steps: initiation, amplification and propagation.

Figure 1. Coagulation factors and coagulation cascade



The extrinsic coagulation pathway and the different coagulation factors that are part of it is illustrated. 1. Tenase complex, 2. prothrombinase complex (adapted from Duodecim Veritaudit, Porkka et al. 2015).

The surface of the vessel wall consists of endothelial cells, preventing contact of circulating blood with the thrombogenic tissue factor (TF) rich inner layers of the vessel wall (Félétou 2011). The endothelial cells do not express TF on their surface, unless stimulated by inflammatory mediators (Félétou 2011). However, plasma factor VII (FVII) can percolate through the endothelium of the vascular wall and contact the TF of the inner layers of the vessel wall. The TF activates FVII and the TF–FVIIa complex produces a small amount of thrombin. This initiation phase of coagulation has been proposed to be constantly active. When injury or inflammation occur to the vessel wall the small amount of thrombin is able to start the amplification phase of the coagulation (Hoffman and Monroe 2001).

The amplification phase occurs as a result of vessel injury when large components of blood such as platelets, vWF and FVIII come into contact with the small amounts of thrombin produced by the initiation phase.

Thrombin activates platelets and FV, cleaves and activates FVIII from vWF multimers. In addition, thrombin activates factor XI, which forms a tenase complex with FVIIIa (Figure 1). vWF has an important role in coagulation as it binds and protects the circulating FVIII from degradation, and localizes and releases FVIII at the site of vascular injury. vWF is also essential for the platelet–vessel wall and platelet–platelet interactions. In particular, vWF enables platelet attachment in the

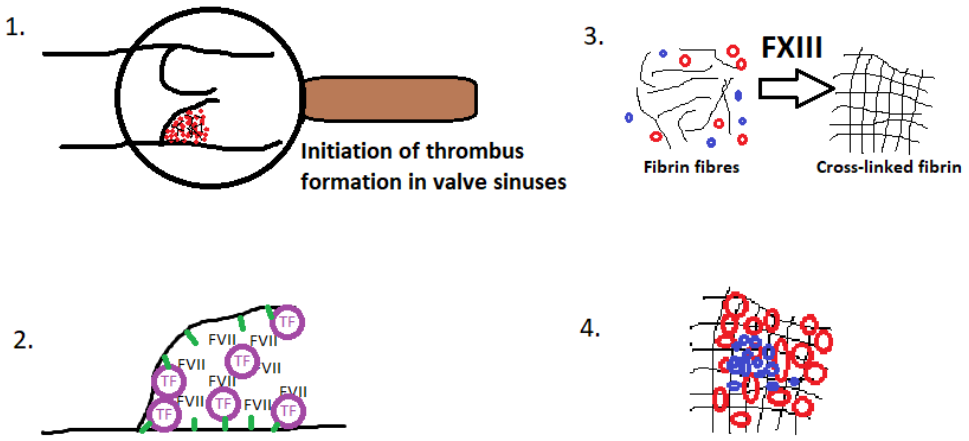
vessel wall under the high shear rate conditions typical of arterial flow (Franchini and Lippi 2006). The tenase complex activates in turn factor X (FX) and propagation of the coagulation follows.

The propagation phase starts when the activated FX and FV form a prothrombinase complex (Figure 1). The rate-limiting component is FXa as FV is both circulating in plasma and secreted from activated platelets leading to a surplus of FVa at the site of thrombosis (Mann et al. 2003). The prothrombinase complex enhances the rate of thrombin production by several orders of magnitude (Nicolaes and Dahlbäck 2002). The thrombin cleaves Fg into fibrin monomers, which are cross linked by FXIIIa to form a netlike structure. The fibrin network enables blood cells, especially red blood cells, to adhere and cover the injury site (Hoffman and Monroe 2001).

6.1.2 Thrombus formation in venous thromboembolism

The activation of the coagulation system in venous thrombosis has somespecific features. The initiation of venous thrombosis is thought to occur in the venous valve pockets of lower extremity veins (Sevitt 1974) (Figure 2). It has been shown that contrast media can linger in the valve sinuses for nearly 30 minutes post venography in supine patients, illustrating a reduced blood flow velocity in these areas (McLachlin et al. 1960). A decrease in partial oxygen pressure in the slowly circulating blood near the valve pockets has been shown in animal and human experiments (Hamer et al. 1981). The hypoxaemia on the endothelial surface mimics injury to the vessel, and accordingly endothelial cells have been shown to localize microparticles rich in TF to the area of hypoxaemia and also start to express TF themselves (Myers et al. 2003, Félétou 2011). When circulating FVII encounters this TF, the stepwise activation of the coagulation cascade essential to the formation of thrombus is initiated and further propagated.

Figure 2. Formation of venous thrombosis



1. Decreased blood flow velocity in valve sinuses causes hypoxaemia in the endothelial cells of vein valves.
2. Hypoxaemia activates endothelium to localize tissue factor (TF) rich microparticles and express TF. TF activates circulating factor VII and initiates the coagulation cascade, which produces fibrin fibres. (Green spikes represent the endothelial cells that localize TF.)
3. Fibrin fibres are cross linked by activated coagulation factor XIIIa (FXIIIa).
4. Cross linked fibrin forms a network on which the blood cells (red circles represent red blood cells and blue dots platelets) attach and thrombus is formed.

6.2 Manifestation and composition of venous thromboembolism

6.2.1 Venous thromboembolism's clinical manifestations

VTE can manifest as PE, DVT or both at the same time. A meta-analysis estimated that in approximately half of the patients with proximal DVT, an embolus in the pulmonary arteries can be detected by imaging (Stein et al. 2010). Also, in half of PE patients there is a coexisting DVT (van Langevelde et al. 2013). The factors regulating this variation are poorly understood.

There is evidence from *ex vivo* experiments that the haemostatic properties of the blood derived from patients with only DVT differ from that derived from PE patients. Two studies with a total of 174 patients reported similar results – the thrombus formed from the plasma of PE patients was more permeable, its density

was lower and the lysis time was shorter compared with DVT patients (Undas et al. 2009, Martinez et al. 2014).

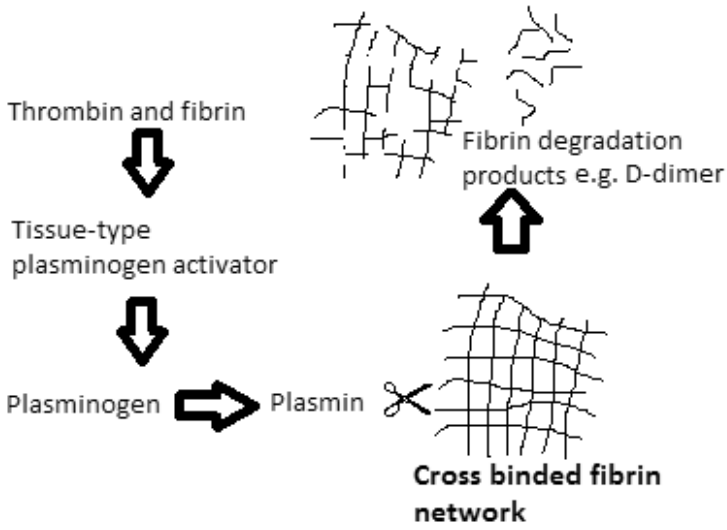
6.2.2 Regulation of thrombus composition and extent

A venous thrombosis consists mostly of fibrin meshwork and red blood cells (Figure 2), but although not so abundant, platelets also have an important role in VTE formation (Sevitt 1974, Chernysh et al. 2020). Activated platelets offer a negatively charged phospholipid surface for coagulation factor complexes, secrete FV and vWF to the site of thrombosis, and stabilize the forming thrombus (Montoro-García et al. 2016).

The fibrin meshwork density can be variable interindividually and there is evidence that coagulation factors XIII and XII affect the density of the meshwork, which in turn affects the amount of attachment of red blood cells to the forming thrombus (Konings et al. 2011, Aleman et al. 2014).

The actions of both natural anticoagulants and the fibrinolytic system (Figure 3) also regulate thrombus formation. The fibrinolytic system is activated by the formed fibrin and thrombin. Plasmin, a key serine protease of the fibrinolytic system, cleaves fibrin and degrades the fibrin network (Chapin and Hajjar 2015). D-dimer, one of the fibrin degradation products, consists of two D fragments of fibrin connected by a cross link, and its elevated levels can be measured during thrombosis as a marker of fibrinolysis. Thus, the interplay of coagulation and the fibrinolytic system regulates the extent of the venous thrombus. However, the exact mechanisms behind the variation of clinical manifestations of VTE are still unknown.

Figure 3. Action of the fibrinolytic system



1. Thrombin and fibrin activate endothelial cells to release tissue-type plasminogen activator.
2. Tissue-type plasminogen activator converts plasminogen to plasmin.
3. Plasmin degrades fibrin.
4. Fibrin degradation products such as D-dimer are released.

A few studies, mainly experiments using either mice or *ex vivo* human plasma, have evaluated the role of various coagulation factors in the regulation of thrombus size. In a study by Aleman, the thrombi formed *in vitro* from the plasma of FXIII-deficient and wild-type mice were compared and it was shown that the thrombi formed from the former were smaller (Aleman et al. 2014). Similar results were seen in a study by Byrnes, where thrombus formation was studied *in vitro* from human plasma and the FXIII function was either inhibited or not. The thrombi formed when FXIII was inhibited were smaller than when the natural function of FXIII was allowed (Byrnes et al. 2015). The association of FXIII and thrombus size was also seen *in vivo* in a work by Kucher in which it was observed that the PL of FXIII antigen measured during acute PE correlated negatively with the obstruction rate in the pulmonary arteries, an indirect way of evaluating the thrombus volume (Kucher et al. 2003).

The complexity of factors affecting the PE extent was illustrated in a study that examined the extent of initial PE and its recurrence. Sixty-three patients with PE recurrence were evaluated and the original size of the PE was categorized into massive or non-massive.

It was seen that the original size predicted quite poorly the size of the recurrence as in 50% of the subjects with originally non-massive PE the recurrence was larger than the original PE, whereas in only 17% of the patients with originally massive PE the recurrence was also massive (Thomas et al. 2017).

6.2.3 Quantifying the size or volume of the venous thromboembolism

The quantification of the extent of VTE is challenging but has aroused some interest. The existing indexes for evaluation of both DVT (Marder score) and PE (Mastora and Qanadli scores) extent are semiquantitative. They classify the location of the thrombus and the obstruction of each specific vein/artery segment and report an individual index value (Marder et al. 1977, Mastora et al. 2003, Qanadli et al. 2001).

Since the coexistence of PE and DVT is common, there is also an interest in the calculation of the total volume of the VTE. The semiquantitative PE and DVT indexes cannot be directly combined, since their values are not comparative. Only a few studies have focused on assessing DVT or PE volume, and all used semiquantitative methods (Table 1). In the work by Ouriel, the volume of the different vein segments in DVT patients was calculated with computed tomography (CT) imaging (Ouriel 1999). Furlan calculated PE volume from CT images with a software program (Furlan et al. 2012) and found a strong correlation with the semiquantitative Mastora score. Existing studies have not tried to quantify the total VTE volume or to combine the semiquantitative PE and DVT volume indexes. The existing semiquantitative indexes for PE or DVT volume are presented in Table 1.

Table 1. Indexes for the evaluation of pulmonary embolism (PE) or deep venous thrombosis (DVT) volume

Semiquantitative method for the evaluation of PE or DVT extent	Focus	Unit (range)	Reference (author)
Marder score	DVT	points (0–40)	Marder et al. 1977
Mastora score	PE	% (0–155)	Mastora et al. 2003
Qanadli score	PE	% (0–100)	Qanadli et al. 2001
Volumetric index	DVT	ml	Ouriel 1999
Clot burden	PE	ml	Furlan et al. 2012

6.3 Risk factors for pulmonary embolism

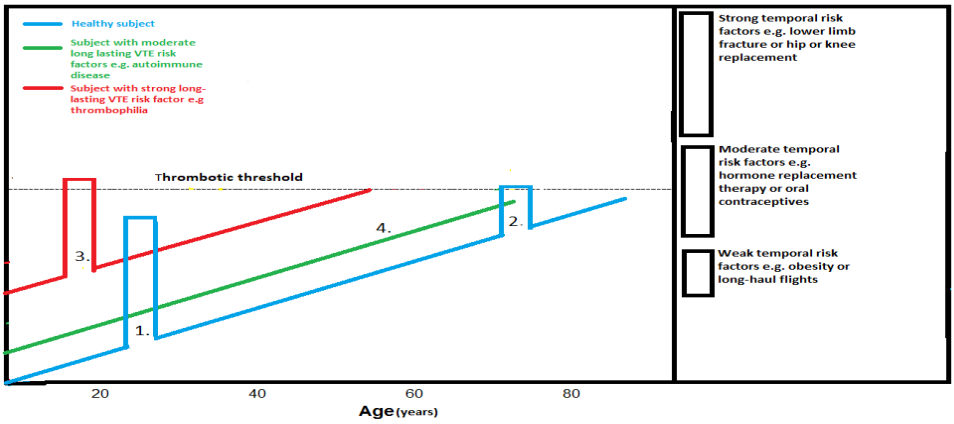
Multiple risk factors for PE have been recognized and often several of these risk factors coexist. Rarely does a single risk factor cause venous thrombosis, rather there is a synergism described as the 'multiple-hit hypothesis' (Esmon 2009). The concept of 1) a thrombotic threshold which varies genetically and with age from person to person and 2) the effect of additional acquired risk factors is depicted in Figure 4. For example, alone obesity or oral contraceptives increase the VTE risk by 1.5- and 4.6-fold, respectively. However, the synergistic joint effect of both increases VTE risk by over 10-fold in obese patients who are using oral contraceptives (Abdollahi et al. 2003).

The latest guidelines of the European Society of Cardiology (ESC) on PE divide VTE risk factors into three classes (Konstantinides et al. 2020) (Table 2). Risk factors are related to one or more aspects of Virchow's triad, which include stasis, hypercoagulability and endothelial injury (Virchow 1856).

Table 2. Risk factors for venous thromboembolism (VTE) according to European Society of Cardiology guidelines on acute pulmonary embolism (Konstantinides et al. 2020)

Risk factors		
Strong	**Moderate**	**Weak**
Previous VTE	Arthroscopic knee surgery	Bed rest >3 days
Lower limb fracture	Autoimmune diseases	Diabetes
Hospitalization for heart failure	Post-partum period	Arterial hypertension
Hip or knee replacement	Hormone replacement therapy or oral contraceptives	Age
Major trauma	*In vitro* fertilization	Obesity
Thrombophilia: phospholipid antigen, homozygous variants	Thrombophilia: heterozygous variants	Pregnancy
	Cancer	Long immobility due to sitting

Figure 4. Illustration of individual venous thromboembolism (VTE) threshold and model for the development of VTE (modified from Rosendaal 1999)



1. Healthy subject can have a strong temporal risk factor such as surgery but the thrombosis does not occur as the thrombotic threshold is not surpassed.
2. The VTE risk increases due to ageing and later a weaker risk factor can cause VTE.
3. A moderate risk factor can cause VTE for patient with higher baseline risk even at a young age.
4. The VTE risk increases with age but the VTE threshold might never be surpassed even if the baseline risk is elevated.

6.4 Pulmonary embolism epidemiology

6.4.1 Pulmonary embolism incidence in the 21st century

The incidence of PE has approximately doubled in the past 20 years and reports from Denmark, England, Germany and the USA show similar development. The incidence was reported to be 89.7 in 2015, 97.8 in 2015, 109 in 2015 and 112 in 2008 per 100,000 inhabitants, respectively (Munster et al. 2019, Kempny et al. 2019, Keller et al. 2020, Wiener et al. 2011).

There are multiple possible explanations for the increased PE incidence. Firstly, the risk factors for VTE are nowadays more common as people are ageing in developed countries and, for example, diabetes has become an epidemic along with obesity (Hu 2011). Also, the incidence of cancer has grown and sophisticated treatment enables cancer patients to live longer (Cancer Society of Finland 2018).
Finally, more medical and surgical procedures are performed than before and also on older patients. For example, hip and knee replacements, which are considered to be strong risk factors, are performed around three times more than 20 years ago in Finland (Finnish Institute for Health and Welfare 2018).

Secondly, more accurate and available diagnostics with computed tomography pulmonary angiography (CTPA) has also likely affected PE incidence. For example, in Finland the use of CTPA increased by 70% from 2011 to 2018 (Säteilyturvakeskus 2019). Due to increased specificity, previously missed subsegmental PEs are now found and it has been shown that the incidence of subsegmental PEs is twofold when multidetector CTPA is used compared with single detector CTPA (Carrier et al. 2010). An earlier report from the USA showed that after the introduction of CTPA in 1998 the incidence of PE nearly doubled in the following 10 years but in the preceding 5 years before CTPA the PE incidence remained at the same level (Wiener et al. 2011). The use of CT for other indications, for example in cancer screening, has also significantly increased and incidental PEs are also commonly found in these screenings (Klok 2017).

6.5 Pulmonary embolism diagnostics

6.5.1 General considerations

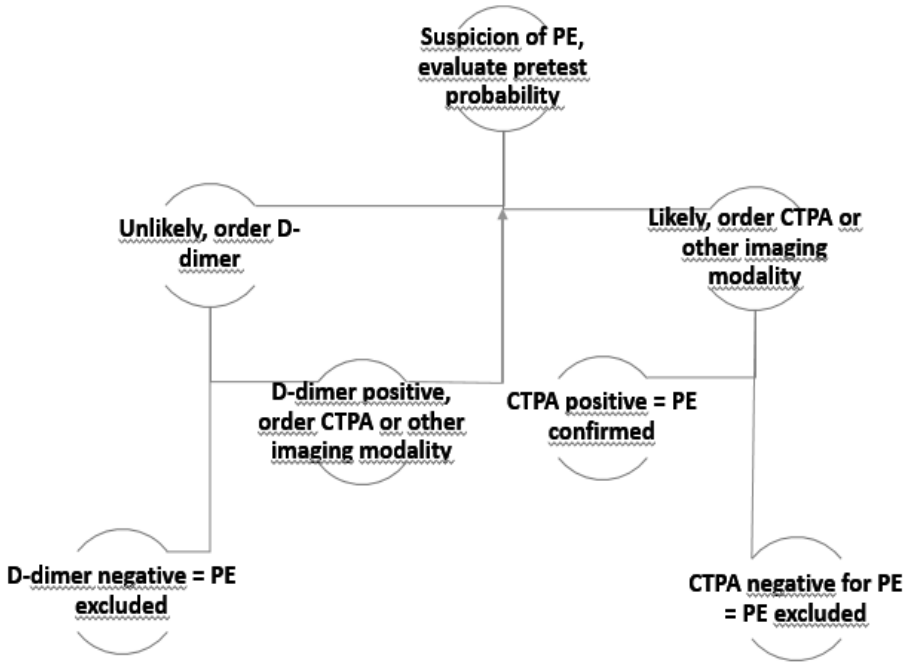
PE diagnostics has advanced significantly during recent decades. The diagnosis of PE includes the patient history, clinical examination, laboratory tests and use of imaging modalities. The diagnosis of PE can be challenging as PE can be asymptomatic and symptoms typical of PE are non-specific and also present in other common illnesses.

The most common clinical symptoms are dyspnoea, tachycardia and pleuritic chest pain (Stein et al. 1991) but these are only present in approximately half of the PE patients. After the suspicion of PE has emerged, the use of diagnostic algorithms to guide the use of imaging studies is helpful, yet clinical judgement can also be used (Figure 5).

In a study with 2400 patients in whom PE was suspected, the clinical parameters and symptoms were similar whether the PE was diagnosed or excluded, illustrating the challenges in PE diagnostics (Pollack et al. 2011).

The timely diagnosis of PE is important for the prognosis. Review of multiple autopsy studies showed that in only one fifth of the subjects with fatal PE was the PE diagnosed ante-mortem (Stein et al. 2016).

Figure 5. Appropriate actions when pulmonary embolism (PE) is suspected in outpatients (modified from the European Society of Cardiology 2019 guidelines, Konstantinides et al. 2020) (CTPA = computed tomography pulmonary angiography)



6.5.2 Clinical decision tools for pulmonary embolism diagnosis

Multiple clinical decision tools (e.g. the Geneva score and the Wells score) (Le Gal et al. 2006, Wells et al. 2000) (Table 3) have been developed during the past 20 years to enhance PE diagnostics. The tools score relevant clinical signs and possible predisposing factors, and calculate the pretest probability before referral studies.

Table 3. Evaluation of the pretest probability of pulmonary embolism (PE)

Item	Original Geneva score (Le Gal et al. 2006)	Item	Original Wells score (Wells et al. 2000)
Previous PE or DVT	3	Previously objectively diagnosed PE or DVT	1.5
Heart rate 75–94 bpm ≥95 bpm	 3 5	Heart rate >100 bpm	1.5
Surgery or fracture within the past month	2	Surgery or immobilization within 4 weeks	1.5
Haemoptysis	2	Haemoptysis	1
Active cancer	2	Active cancer	1
Unilateral lower limb pain	3	Clinical signs of lower extremity DVT	3
Pain on lower limb deep venous palpation and unilateral oedema	4	Alternative diagnosis less likely than PE	3
Age >65 years	1		
Clinical probability Low Intermediate High	 0–3 points 4–10 points ≥11 points		 ≤2 points 2–6 points >6 points
Proportion (%) of confirmed PEs of all suspected cases if pretest probability was: Low* Intermediate* High*	 8 28 74		 3.4 27.8 78.4
*Acquired from the original publications DVT = deep venous thrombosis			

The definition of the pretest probability is also important for identification of those high-risk patients for whom immediate initiation of anticoagulation is warranted. In patients with moderate or high pretest probability the anticoagulation should be started before diagnostic imaging and is shown to reduce mortality (Smith et al. 2010, Kline et al. 2007). However, it should be highlighted that the decision tools were created for outpatient use only.

6.5.3 D-dimer test

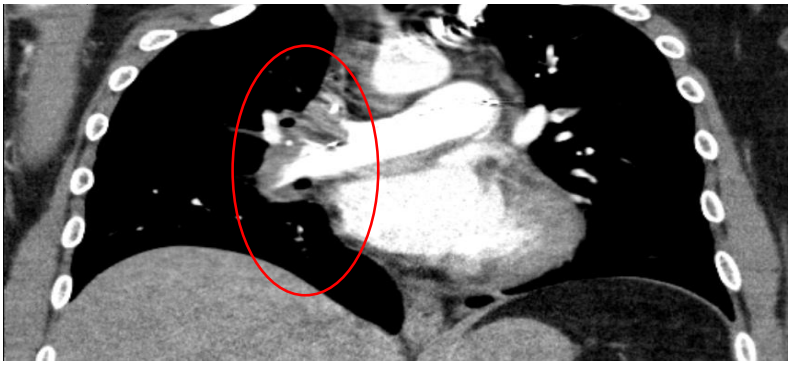
The most important laboratory test for low pretest probability patients is the D-dimer test (Wakefield et al. 2008) (Figure 3). D-dimer is constantly present in the blood at low levels but the elevation of D-dimer above the normal reference range has been indicative of VTE (Weitz et al. 2017(B)). However, it should be remembered that many conditions such as cancer, recent surgery, infection, chronic inflammation, liver or renal disease, and advanced age are associated with increased D-dimer levels (Weitz et al. 2017 (B)).

The universal reference range in plasma is considered to be under 500 ng/ml (HUSLAB 2021), although age-related reference values have also emerged (see chapter 6.5.5). Analysis of D-dimer is based on reaction with monoclonal antibodies and multiple different test types are available, for example ELISA, whole blood or latex agglutination assays. The sensitivity and specificity of different D-dimer assays are at best 95% and 83%, respectively (Weitz et al. 2017(B)). Multiple reasons within assay technical issues or in the qualities of the thrombosis may explain why there is a small possibility that D-dimer is false negative. Thus D-dimer should not be used to exclude PE in patients with high or moderate pretest probability (Frost et al. 2003).

6.5.4 Computed tomography pulmonary angiography

The gold standard for PE diagnostics is CTPA (Konstantinides et al. 2020). The use of contrast media is recommended because the embolus can be iso-attenuated with the circulating blood (Stein et al. 2006). Currently, with multidetector CTPA even subsegmental arteries with a diameter of only 2–3 mm can be evaluated. An example of positive CTPA for PE is shown in Figure 6.

Figure 6. Pulmonary embolism (PE) in the right proximal pulmonary artery



The PE is circled in red and is shown as a grey mass located in the right pulmonary artery bifurcation. The contrast media is shown as white and is present in the right ventricle and in the truncus of the pulmonary artery.

CTPA examination can also be indeterminate for multiple reasons such as patient motion, large body size and suboptimal enhancement of pulmonary arteries with contrast media (Wittram 2007). The main concern is the possibility of false positive diagnosis, which is emphasized in the case of small emboli in the distal subsegmental arteries, where up to 75% of PE diagnoses have been shown to be false positive findings in later analysis (Stein et al. 2006, Hutchinson et al. 2015).

6.5.5 Challenges in pulmonary embolism diagnostics in recent decades

At the beginning of the 21st century, PE diagnosis was still mainly based on findings in ventilation-perfusion lung scintigraphy (V/Q scan), the history of which dates back to the 1960s. This diagnostics modality has its challenges including the longer duration and over quarter of the scans being non-diagnostics (Yazdani et al. 2015). Fortunately, advances were made and for the first time in 1992, spiral CT was shown to have excellent concordance with pulmonary angiography. Higher sensitivity and specificity with the possibility of providing alternative diagnosis explained why CTPA became the gold standard for PE diagnostics and why the transition from the use of V/Q scan to CTPA occurred fairly quickly. In addition, clinical decision tools and the D-dimer test emerged at the beginning of the century and due to these advances the risk of missed PE cases likely decreased, leading to an increase in PE incidence.

However, the more available non-invasive diagnostics tools have also caused the paradigm of the diagnosis to be shifted from diagnosing PE in high-risk patients to the exclusion of PE from low-risk patients (Righini et al. 2017).

The number of patients in whom PE is suspected has increased enormously and thus the proportion of patients in whom PE is diagnosed has decreased to 5% compared with nearly 50% in the 1980s (Le Gal and Bounameaux 2004). In order to address this problem, a number of new modifications to the diagnostic algorithms have emerged. The main idea of these modifications has been the elevation of the D-dimer threshold for the referral studies. It has been presented that the age of the patients should be considered when interpreting the D-dimer results, allowing a higher threshold for older patients (Righini et al. 2014).

Additionally, a new decision tool named 'YEARS' was studied. It was shown that by using the 'YEARS' tool instead of the Wells score the need for CTPA examinations decreased by 13% without increasing the rate of missed PEs (van der Hulle et al. 2017). The effect was mainly because a threshold of 1000 ng/ml versus a standard 500 ng/ml for D-dimer was used in patients in whom the clinical probability of PE was low. The most recent work also showed that PE could be safely excluded if thresholds of 1000 ng/ml for low and 500 ng/ml for moderate pretest probability patients evaluated with the Wells score were used (Kearon et al. 2019). In addition, with these studies a simple strategy of requiring the evaluation of pretest probability before ordering CTPA has been shown to increase the yield of CTPA (Walen et al. 2016).

6.6 Pulmonary embolism treatment and its effect on mortality

6.6.1 Acute treatment

After the PE diagnosis is confirmed, anticoagulation should be started. The evaluation of clinical parameters, laboratory markers and findings from the CTPA helps to determine the risk of early mortality as indexes using standardized information on these parameters have been developed.

The pulmonary embolism severity index (PESI) and its simplified version (SPESI) predict the risk of early mortality of PE patients (Aujesky et al. 2005, Jiménez et al. 2010) and the latest guidelines recommend use of these risk stratification tools (Konstantinides et al. 2020).

PE patients in acute settings can be categorized into three groups. Approximately 5%, 40–50% and 50% of the patients have high (on average 25%), intermediate (5%) or low (<1%) early mortality risk, respectively (Keller et al. 2020, Aujesky et al. 2005). The use of thrombolysis is recommended for high mortality risk patients

(e.g. with unstable haemodynamics) to improve survival (Stein et al. 2012), and surveillance in an intensive care unit is appropriate. Additional options for high-risk patients are surgical or mechanical embolectomy (Konstantinides et al. 2020).

The use of thrombolysis for intermediate-risk patients is contraindicated as it has been shown to increase bleeding complications without clear benefit to PE mortality (Meyer et al. 2014). Hospitalization is appropriate as the risk of haemodynamic collapse is 5% in the 48 hours following diagnosis.

Low-risk patients may be hospitalized but as the risk of mortality is low in the light of latest evidence these patients can also be discharged after PE diagnosis as long as proper outpatient care with anticoagulant treatment can be provided (Barco et al. 2020 (A)).

The prognosis of PE patients after diagnosis and the initiation of appropriate treatment is generally good as the 30-day PE-related mortality has been shown to be only 1.7% in a large registry study (Jiménez et al. 2019).

6.6.2 Anticoagulation

Anticoagulation, and its early initiation, reduces overall PE mortality and VTE recurrence (Konstantinides et al. 2020). Several anticoagulation options have emerged during the last decade. The traditional standard treatment in the 1990s, 2000s and early 2010s was most often initiated with low-molecular-weight heparin (LMWH, inactivator of FXa) (Konstantinides et al. 2020), followed by vitamin K antagonist an an important cofactor in the production of coagulation factors II (FII), VII (FVII), IX (FIX) and X (FX) (Suttie 1969). However, in the last decade new options of direct oral anticoagulants have emerged and they have replaced the use of standard therapy (Wändell et al. 2019). These novel drugs have an effect by either directly inhibiting thrombin or activated FX.

Sufficient duration of anticoagulation is considered to be 3 months in most cases. Predisposing factors influence the recurrence risk of PE. PEs with strong risk factors have low recurrence rates, whereas PEs with no or only mild predisposing factors recur more often. The prevention of recurrence might decrease PE mortality, and the latest guidelines suggest that lifelong anticoagulation already after the first VTE for patients with no or only weak predisposing risk factors should be considered (Konstantinides et al. 2020).

6.7 The Prognosis of pulmonary embolism

6.7.1 Risk and significance of pulmonary embolism recurrence

The risk of recurrence is affected by the aetiology of the original PE. Traditionally, PE patients were classified as having idiopathic or secondary PE but the latest ESC guidelines expand this categorization into three groups, where PE can be caused by either no or weak, moderate or strong risk factors (Konstantinides et al. 2020) (Table 2). The VTE recurrence risk is estimated to be 1, 3–8 or >10% annually for patients with either strong, moderate, weak or no predisposing factors, respectively (Iorio et al. 2010).

The risk of recurrence is noteworthy but as important are the consequences of the recurrence. The actual risk of fatal recurrence is low and this result has been verified in different settings, for example in a large cohort study with 2000 patients (Douketis et al. 2007), a population-based registry study with 3500 PE cases (White et al. 2008) or a meta-analysis of clinical studies with 7500 patients (Khan et al. 2019). In these studies, the overall yearly incidence of fatal recurrence of PE was approximately 0.3% and the case fatality rate in PE recurrence was estimated to be 3.8%.

PE can also cause long-term consequences and the rare disease entity of chronic thromboembolic pulmonary hypertension can develop in approximately 3% of PE survivors (Ende-Verhaar et al. 2017).

6.7.2 Mechanism of fatal pulmonary embolism

When the embolus obstructs the pulmonary arteries the PE does not only cause mechanical obstruction but also induces vasoconstriction by releasing vasoconstrictive mediators (Niden and Aviado 1956). Neurohormonal activation and systemic vasoconstriction ensure that despite high pulmonary artery pressure the blood flow in the pulmonary arteries is preserved, but this also leads to dilatation of the right ventricle (Konstantinides et al. 2020). Gas exchange in the lungs is also disturbed as there is a mismatch of ventilation and perfusion in the lungs (Burrowes et al. 2011). The dilatation of the right ventricle impairs the filling of the left ventricle, thus leading to dyssynchrony and a decrease in cardiac output. Haemodynamic collapse can follow and fatality in these patients is high even with advanced care (Konstantinides et al. 2020).

6.7.3 Mortality caused by pulmonary embolism

PE can have a fatal outcome and by evaluating the mortality statistics in a population the death toll from PE can be calculated. PE mortality is defined as the proportion of all PE deaths divided by the number of people in the population, but challenges exist when PE mortality is evaluated. The role of PE in the chain of events leading to death may not always be straightforward especially if death follows late after the diagnosis, and the ante-mortem recognition of PE as a cause of death may also be challenging as the symptoms of PE are diverse. As a consequence the standards of post-mortem cause of death evaluation impact to the statistics.

PE commonly presents with illnesses which independently have a bad prognosis; for example, with active cancer and up to 20% of PE patients are dead a year after the diagnosis (Klok et al. 2010). The determination of whether PE or predisposing illness caused the death is a matter of debate.

In Finland, the death certificate offers the possibility to define immediate, underlying and contributory causes of death and PE can be listed as the immediate and underlying cause of death when it is the most likely illness to have caused the death. However, this is only if there is no other illness predisposing to PE that could be defined as the underlying cause of death. If PE is considered to have only an adverse effect on overall health, it can be defined as a contributory cause of death.

The practice patterns of cause of death evaluation in different countries likely influence whether and when PE is listed as an immediate, underlying or a contributory cause of death, as illustrated in a recent study from the European region, where an over 10-fold difference between countries in PE mortality incidence was seen when individuals with PE or DVT listed as the underlying (primary) cause of death were evaluated.

As the definition of PE death can be challenging, PE mortality has been mainly analysed post-mortem by including all the cases where PE was listed among the causes of death, and this method may better illustrate the true burden of PE.

In Finland, the majority of PE deaths when evaluated from a population cohort of 1.7 million in four consecutive years occurred suddenly and were only diagnosed post-mortem in autopsy (Sane unpublished results). Consequently the autopsy rate and changes in autopsy rate or customs surrounding autopsies likely influence the incidence and reliable evaluation of PE mortality as the majority of PE deaths are only diagnosed post-mortem.

Despite the challenges in evaluating PE mortality, real-life data have shown that PE mortality has decreased in almost all population-based studies evaluating PE mortality in the 21st century (Table 4). It seems that the prognosis of PE has improved in recent decades as PE mortality has decreased uniformly in different geographical locations.

Table 4. Main findings of the existing nationwide studies on pulmonary embolism (PE) mortality in the 21st century

Study location	Study period	How PE mortality was defined	PE mortality/ 100,000 years at the end of the study period	Main finding	Parallel change in autopsy rate
Australia (Shiraev et al. 2013)	1997–2007	Not clarified, most likely only PE as underlying cause of death analysed	1.73	PE mortality decreased 21% during study period	Not available
USA (Wiener et al. 2011)	1998–2008	All cases where PE was among the causes of death were included	11.9	PE mortality decreased 3% during study period	Minor increase after 2003
France (Olié et al. 2015)	2000–2010	All cases where PE was underlying or contributory cause of death	6.6 for PE as underlying, 13.5 for PE as contributory	PE mortality decreased 30% during study period when overall PE mortality was analysed	Not available
European region (Barco et al. 2020 (B))	2000–2015	PE or DVT as underlying/primary cause of death	6.5 (range 1.2–24)	PE mortality decreased 49% during study period	Decreased: available only for minor proportion of the countries
USA (Martin et al. 2020)	1999–2017	All cases where PE was underlying or contributory cause of death	3.5 for PE as underlying, 14.4 for PE as contributory	PE mortality decreased 30% during study period when PE as underlying cause of death was evaluated	Minor increase after 2003

DVT = deep venous thrombosis

7. Aims

The aims of this thesis were to 1) clarify the connections between PLs of haemostatic markers and the features of acute PE, and 2) to assess the long-term trends in PE mortality in Finland.

The specific aims of the studies were to:

1. Investigate PE-induced changes of the PLs of haemostatic markers by comparing the acute phase with the stable phase after PE subsidence and also with the levels of healthy controls.

2. Evaluate the association and correlation of patient characteristics and PLs of haemostatic markers with the volume of VTE.

3. Assess how PE patients with or without coexisting DVT differ in terms of patient characteristics and PLs of haemostatic markers.

4. Report PE mortality data from Finland in the past 20 years and evaluate the association of autopsy rate with PE mortality.

8. Methods

8.1 Study cohorts

8.1.1 Studies I–III

For studies I to III, 63 consecutive patients with first ever PE were enrolled between February 2003 and August 2004 in the Emergency Department of Helsinki University Hospital. PE was confirmed by CTPA. Additionally, a total of 15 age- and gender-matched healthy individuals (voluntary co-workers and relatives of the researchers) served as controls.

8.1.2 Study IV

The study population was collected by utilizing national registry data. The term PE deaths covers all the deaths where PE was defined either as an immediate, underlying or a contributory cause of death. PE mortality stands for all PE deaths divided by the number of people in the population, and an incidence of 1:100,000 years is reported.

The number of PE deaths from 1996 to 2017 was collected from the death certificate archive maintained by Statistics Finland. Information on the deaths occurring in Finland or abroad of persons permanently resident in Finland is stored in the death certificate archive. Death certificates are mandatory to complete for all those deceased, and diagnosis of an illness as the underlying cause of death or the immediate cause of death, together with possible contributory causes of death, is defined. An underlying cause of death is a disease that initiated a series of illnesses leading directly to death or the circumstances connected with the accident that caused an injury leading to death. An immediate cause of death is a disease whose symptoms most likely caused a death. A contributory cause of death is a disease that adversely affected the development of the condition leading to death. Altogether, five contributory causes of death can be postulated in the death certificate. Data for all the subjects with International Statistical Classification of Diseases (ICD) code I26.0 or I26.9 for PE as their immediate, underlying or contributory cause of death were collected and in total 25,163 patients with PE death were identified.

8.1.3 Additional informationfor assessing pulmonary embolism incidence

The number of patients diagnosed with PE in Finland from 1997 to 2017 was collected from the Care Register for Health Care managed by the Finnish Institute for Health and Welfare. The Care Register for Health Care collects social security code linked information of both inpatient and outpatient use of health services. Data on all the subjects with ICD code I26.0 or I26.9 for PE as their hospital discharge diagnosis were collected and in total 100,537 subjects were identified.

8.2 Study design

8.2.1 Studies I–III

Studies I, II and III were based on an observational study conducted on 63 consecutive PE patients (33 female, mean age 56.5 years) (Table 7) who were recruited from Helsinki University Hospital between 2003 and 2004, and 15 age- and gender-matched healthy controls. Study recruitment took place after PE diagnosis was confirmed in CTPA.

The exclusion criteria were: history of previous PE, preceding use of anticoagulation therapy, high-risk PE with unstable haemodynamics, diagnosis of asthma or chronic obstructive pulmonary disease, pregnancy, previous diagnosis of heart failure, unstable angina pectoris, and terminal cancer with life expectancy of less than 6 months.

8.2.2 Study IV

Study IV was a registry-based trial using population-based information on death caused by PE and the autopsy rate of the population. A cohort of patients with PE deaths included all the patients from 1996 to 2017 with ICD code I26.0 or I26.9 for PE listed among the causes of death.

During data gathering the following registries were utilized:

- The death certificate archive provided by Statistics Finland. This registry enables social security code linked identification of patients with specific diagnoses in their death certificates.
- The StatFin open database was used to evaluate the number of autopsies, the most common underlying causes of death in the population and population demographics (Statistics Finland 2020).

8.3 Modalities and methods used

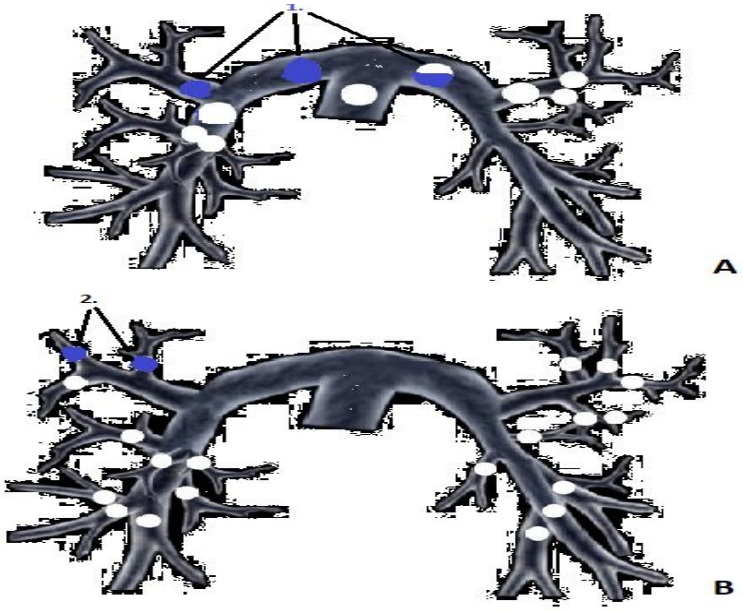
8.3.1 Imaging of pulmonary embolism (studies I–III)

PE patients were recruited after PE had been diagnosed. Diagnostics and treatment were performed according to clinical judgement of the treating physician and by utilizing the guidelines of that time. PE was diagnosed with CTPA in all patients. Thirty-six examinations were performed with an 8-slice scanner, 22 with a 4-slice scanner and five with a single-slice scanner. Slice thickness was 1 or 1.25 mm in the multi-slice scanners and 3 mm in the single-slice scanners. The volume of the contrast material varied between 90 and 120 ml and it was injected with a power injector using bolus tracking. All CTPA scans were centrally assessed by two expert radiologists blinded to the other's results.

8.3.2 Defining the extent of pulmonary embolism (studies II and III)

From the patients included in the study the obstruction of the pulmonary arteries was evaluated by using the method of Mastora (Mastora et al. 2003). The Mastora score is a semiquantitative method for evaluating the PE mass. It incorporates obstruction evaluation of the proximal (five mediastinal and six lobar arteries) and peripheral pulmonary arteries (20 segmental arteries). The obstruction of each artery is evaluated by using a 5-point scale: 1: <25%, 2: 25–49%, 3: 50–74%, 4: 75–99% and 5: 100% obstruction. The total obstruction is calculated by summing the obstruction of each individual artery, and the maximum obstruction is 155 (11 proximal × 5 and 20 segmental arteries × 5) (Figure 7).

Figure 7. Illustration of pulmonary arteries and Mastora score



1. Figure A shows the evaluated proximal pulmonary arteries and total obstruction of the right pulmonary artery, 50% obstruction of the left pulmonary artery and total obstruction of the right upper lobar pulmonary artery in an individual example. Total central Mastora score 12.5 (5 points for the right pulmonary artery, 2.5 points for the left pulmonary artery and 5 points for the right upper lobar artery).
2. Figure B shows the evaluated peripheral pulmonary arteries and total obstruction of two right upper segmental arteries. Total peripheral Mastora score 10 (5 points for each obstructed right upper segmental pulmonary arteries).

8.3.3 Imaging of deep venous thrombosis (studies I–III)

The presence of coexisting DVT was evaluated in every patient with bilateral lower extremity duplex ultrasound with a standard 10 MHz linear array probe by the consultant radiologist. The protocol for the ultrasound examination included compression of the lower extremity veins including: common femoral, superficial femoral, popliteal, posterior tibial and peroneal veins. Compression of the entire leg was imaged at 3 cm intervals. Additionally, Doppler ultrasound was performed at the saphenofemoral junction to assess the existence of more proximal venous thrombosis. If DVT was detected an exact anatomical location and extent was reported.

8.3.4 Calculation of venous thromboembolism volume (study II)

The PE volume was estimated by using the Mastora score of the patients and equalizing the score with a volume calculation model presented by Furlan (Furlan et al. 2012). In this model, a dedicated semi-automated software program was used to define the clot volume in cubic millimetres, a volume which strongly correlated with the Mastora score. In the proximal and peripheral arteries, a 1% Mastora score was equal to 0.36cm^3 and 0.17 cm^3 of thrombus, respectively. Originally, the software was tested with both PE phantom and 30 PE patients with two observers in two separate sessions. The intra- and interobserver agreement between measurements was excellent, illustrating that this method is feasible and reliable in quantitative PE volume analysis (Furlan et al. 2011).

DVT location and extent were defined by compression ultrasound and approximating the volume on the basis of the data from a study by Ouriel (Ouriel 1999). Ouriel determined the volume of the different segments of lower extremity veins by using CT, ultrasound and venography in defining the diameters and lengths of the veins. By utilizing the information on average volumes of different vein segments in lower extremities and using simple arithmetic we were able to calculate the volume of the thrombosis. For example, if the thrombosis extended from the whole popliteal vein to half of the superficial femoral vein the DVT volume would be 7.9 + (14.6 / 2) = 15.2 cm^3 (Ouriel 1999).

8.3.5 Exclusion of chronic lung pathology (study III)

Patients with known chronic pulmonary diseases were excluded based on the sub-study in which PE effect on pulmonary function was evaluated (Piirilä et al. 2011) and additionally to prevent the possibility that change in pulmonary function was caused by chronic lung disease. The presence/absence of bronchial inflammation was analysed by measuring the fractional exhaled nitric oxide (FENO). FENO yields the nitric oxide concentration from exhaled air, which is a surrogate marker for eosinophilic inflammation of the bronchial tree and was used to ensure that patients with previously undiagnosed asthma would be identified.

8.3.6 Controlling confounding factors related to pulmonary embolism mortality (study IV)

The number of autopsies was collected from Statistics Finland's StatFin open database (Statistics Finland 2020). The annual number of autopsies is reported for both genders separately and these data are available from 1976. The number of all deaths was also collected from this database to calculate the autopsy rate.

The most common illnesses defined as the underlying cause of death were collected from the StatFin open database from the total population and subjects with PE death. Changes during the follow-up periods in these two cohorts were compared.

Ageing of the population has an impact on PE incidence and mortality statistics. Population statistics from Finland were also collected from the StatFin open database and changes per age quartile were evaluated. The standard population during the study period for age adjustment was calculated as follows. The annual number of subjects in each 5-year age group were summed together and divided by the number of follow-up years to define the standard population corresponding to the average population during the follow-up period. The age-adjusted PE mortality was also reported.

8.4 Laboratory analysis of haemostatic markers (studies I–III)

8.4.1 Choice of analysed markers and reference values

The analysed haemostatic markers were chosen on the basis of the following: 1) they reflect various phases of the clotting process and/or related inflammatory response; 2) their assessment is not affected by anticoagulation treatment. Anticoagulation was started before randomization, and the choice of the long-term treatment (LMWH or vitamin K antagonist) was made by the treating clinician irrespective of this study. The analysed haemostatic markers and their normal range are presented in Table 5.

Table 5. Investigated haemostatic and other markers and their reference ranges

Analysed marker	**Normal range (HUSLAB 2021)**
D-dimer	<0.5 mg/l
Fibrinogen	2–4 g/l
Factor V	65–140%
Factor VIII	60–160%
Factor XIIIa	76–156%
Soluble thrombomodulin	24–57 ng/ml*
von Willebrand factor antigen	0.5–1.9 IU/ml
C-reactive protein	<4 mg/l
Red blood cell count	Male 4.25–5.7 E12/l Female 3.9–5.2 E12/l
Platelet count	150–360 E9/l
*In 15 healthy controls	

8.4.2 Blood collection and storage

Collection of the samples was standardized. Blood was collected via venepuncture from the cubital vein with a 20-gauge needle (Venoject® Terumo Medical Corporation, NJ, USA) in 3.2% sodium citrate (9:1, v/v) tubes. The first 5 ml of the blood was discarded. During the collection process, no plastic instruments containing polypropylene were used.

8.4.3 Laboratory analysis

Analysis of the samples was performed in Helsinki University Hospital's central laboratory and in the Department of Haemostasis of the Finnish Red Cross Blood Service. During the study period, the methods used remained unchanged in both laboratories and there was no significant assay drift.

Analysis of D-dimer, red blood cell count, platelet count, C-reactive protein (CRP), FV activity and FVIII activity was done within a few hours if the PE diagnosis was made during office hours. Otherwise, the samples for FV activity and FVIII activity were stored at −80°C and analysed the next day. The samples for FXIIIa, thrombomodulin and vWF:Ag were analysed twice a week after storage at −80°C.

Sample collection in the stable phase and for the healthy controls was during office hours and managed accordingly.

Specimens for D-dimer, Fg, FV, FVIII and FXIIIa were centrifuged for 10 minutes at 2000 g and 4°C, and those for vWF:Ag and thrombomodulin for 10 minutes at 1800 g and 4°C.

D-dimer, red blood cell count, platelet count, CRP, FV activity and FVIII activity assays were performed according to standard hospital protocol. The FXIIIa, thrombomodulin and vWF:Ag assays were processed by a laboratory technician skilled in coagulation analysis. Details of various available coagulation assays are summarized in Table 6.

Table 6. Coagulation assays used in studies I–III

Assay screening test	Manufacturer	Reagent	Reference range	Used in studies	Special notes
D-dimer	Roche Diagnostics	Tina-Quant D-Dimer	<0.5 mg/l	I–III	
Fibrinogen	Diagnostica Stago	STA®-Fibrinogen 5	1.8–3.4 g/l	I–III	
Factor V	Siemens Healthcare Diagnostics	Dade w Innovin w	79–139%	I–III	
Factor VIII	Siemens Healthcare Diagnostics	Pathromtin SL	59–131%	I–III	
Factor XIIIa	Dade Behring	Berichrom FXIII	64–154%	I–III	*
von Willebrand factor antigen	Diagnostica Stago	STA-Liatest® vWF:Ag reagent	0.51–1.62 IU/ml	I–III	*
Thrombomodulin	Diagnostica Stago	STA-Liatest®	**	I–III	*
Red blood cell count	Sysmex 4500 haematology analyser		3.9–5.7E 12/l	II	
Platelet count	Sysmex 4500 haematology analyser		150–360E 9/l	II	

* FXIIIa, thrombomodulin and vWF:Ag: combined CV 3.6% for the low FXIIIa and 2.6% for the normal FXIIIa
** Not available

8.5 Venous thromboembolism risk factors and screening for thrombophilia

Patient characteristics, medical history and medication used were thoroughly investigated. For example, the following clinical risk factors were evaluated: preceding immobilization, body mass index (BMI), smoking, diabetes, previous DVT and preceding use of hormone replacement therapy or oral contraception. Additionally, symptom duration information and the use of antiplatelet medication were evaluated.

Thrombophilia screening was performed for all patients and it included measurement of proteins C and S and antithrombin activity. FV Leiden and prothrombin mutations were also assessed. The existence of antiphospholipid antigens was also investigated.

8.6 Ethical aspects

The study design (studies I–III) was approved by the Ethics Committee of the Helsinki University Hospital and written informed consent was obtained from all participants. For study IV the ethics committee decided that ethical permission was not needed as the study was based on exploring register data.

8.7 Statistical analysis

For the recorded data in all studies, mean values and standard deviation were calculated. The normality of continuous variables was checked by the Kolmogorov–Smirnov test. All of the statistical analyses were performed with SPSS 21.0 for Windows (IBM Corp., Armonk, NY, USA). A *p*-value of <0.05 was considered statistically significant.

8.7.1 Studies I–III

Differences in the haemostatic markers in the acute and stable phases were assessed with the Wilcoxon signed-rank test and the paired samples *t* test. The independent samples *t* test was used in the analysis of the differences between healthy controls and PE patients as well as in evaluating the differences in haemostatic markers in patients with or without LMWH or warfarin during measurement collection.

Correlation of the haemostatic markers with VTE volume was analysed with the univariate Pearson's correlation test. The independent samples *t* test was used when analysing the differences in VTE volume in patients with or without evaluated risk factors. One-way analysis of variance was used to determine differences in VTE volume in patients with distal, proximal or no DVT.

The independent samples *t* test was used to analyse the differences in haemostatic markers between PE patients with or without coexisting DVT. Cross-tabulation and Fisher's exact test were used to evaluate the impact of the dichotomically categorized variable of PE location on DVT coexistence as well as a thrombophilic effect on DVT existence in PE patients.

8.7.2 Study IV

The crude PE mortality rate was calculated by dividing the annual number of PE deaths by the mid-interval total population (deaths per 100,000 population). The age-standardized PE mortality based on 5-year age groups was obtained from the population statistics of Finland and presented in age quartiles. Linear regression was used to test the statistical significance of the changes in PE mortality in relation to time as well as the association between the number of autopsies and PE deaths, the changes in PE mortality for males and females, and in periods of unchanged or decreasing autopsy rate in 1996–2009 and 2010–2017. In addition, the changes in proportion of the most common underlying causes of death in subjects with PE death and in the population were evaluated with linear regression. We express the change in PE mortality as an average annual percentage change (AAPC) calculated as the average of individual annual changes. The independent samples *t* test was used to evaluate the difference in clinical characteristics of the female and male subjects. The chi-square test was used to evaluate the difference in autopsy rates between 1996 and 2017. A *p*-value of <0.05 was considered statistically significant.

9 Results

9.1 Studies I–III

9.1.1 Patient characteristics in studies I–III

Sixty-three PE patients (33 females) were investigated (Table 7).

Table 7. Demographic and clinical characteristics of the study population in studies I–III

Variable	**Patients, n=63 (%)**	**Healthy controls, n=15 (%)**
Age (years, range)	56.5 (18–86)	55.2 (24–76)
Gender (female/male)	33/30	9/6
Body mass index (kg/m^2, range)	29.5 (20.9–42.8)	(Normal weight)
Diabetes	5 (8)	0 (0)
Smoking - Smoker - Ex-smoker - Non-smoker	 11 (18) 19 (30) 33 (52)	 15 (100)
Risk factors for VTE - Immobilization - Hormone replacement therapy - Hormonal contraception - History of DVT - Family history of VTE - A heterozygous factor V variant - Cancer	 28 (44) 7 (11) 6 (10) 7 (11) 5 (8) 5 (8) 3 (5)	
Antiplatelet medication (yes/no)	15/48	
DVT = deep venous thrombosis VTE = venous thromboembolism		

9.1.2 Haemostatic markers in the acute and stable phases of pulmonary embolism, and in healthy controls

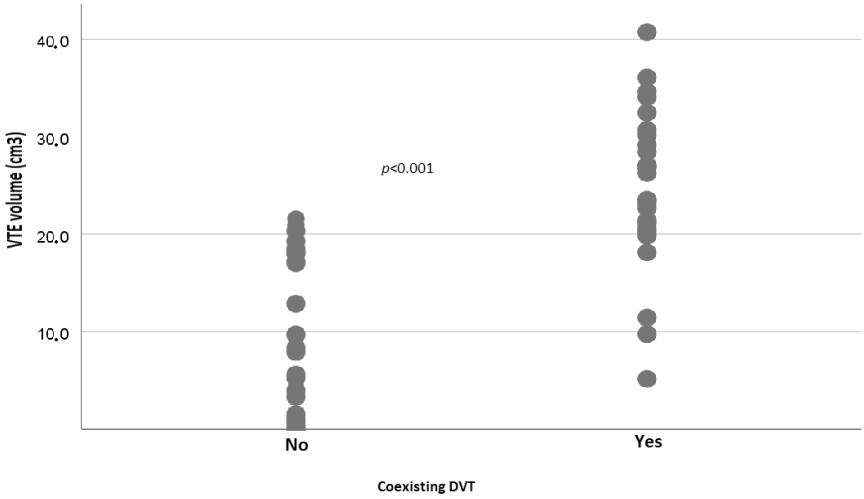
In PE patients, the PL of FXIIIa was significantly lower in the acute phase compared with the stable phase (87.5% vs 117.7%, *p*<0.001), and the stable phase PL of FXIIIa was similar to that of the healthy controls (117.7% vs 109.0%, *p*=0.2) (Table 8). The PLs for vWF:Ag and FVIII were higher in the acute phase than in the stable phase (2.66 IU/ml vs 2.01 IU/ml, *p*<0.001, for vWF:Ag, and 167.2% vs 155.1%, *p*=0.07 for FVIII). The vWF:Ag value remained higher in the stable phase compared with healthy controls (2.01 IU/ml vs 1.43 IU/ml, *p*<0.01). D-dimer was above the reference threshold in all patients (range 0.7–31.8 mg/l) in the acute phase and after the treatment period it was under the threshold in 52 patients (80%).

Table 8. Plasma levels of haemostatic markers of pulmonary embolism patients in the acute and stable phases and in healthy controls

Haemostatic markers	**Acute phase**	**Stable phase**	**Healthy controls**	***p*-value for the difference (acute and stable phases)**	***p*-value for the difference for PE patients in stable phase and healthy controls**
D-dimer (mg/l)	8.9 (7.6)	0.7 (0.9)	NA	<0.001	
Factor XIIIa (%)	87.5 (25.0)	117.7 (31.6)	109.2 (20.3)	<0.001	0.2
von Willebrand factor antigen (IU/ml)	2.65 (1.21)	2.01 (0.84)	1.42 (0.51)	<0.001	<0.01
Factor VIII (%)	167.2 (55.9)	155.1 (41.3)	NA	0.07	
Fibrinogen (g/l)	4.3 (1.4)	3.9 (1.0)	NA	<0.05	
Factor V (%)	110.8 (22.3)	110.2 (23.5)	NA	0.9	
Soluble thrombomodulin (ng/ml)	36.6 (12.7)	47.5 (18.4)	37.7 (9.2)	<0.001	0.05
Mean (standard deviation) NA = Not available					

9.1.3 Venous thromboembolism volume (study II)

VTE volume (a combination of the PE and DVT volume) was calculated as explained in chapter 8.3.4. There was large interindividual variation in the mean VTE volume both in patients with and without coexisting DVT (Figure 8).Figure 8. Distribution of venous thromboembolism (VTE) volume in patients with and without coexisting deep venous thrombosis (DVT)



A coexisting DVT was diagnosed in 31 out of 63 PE patients and the difference in VTE volume between these two patient groups was observed (Table 9).

Table 9. Mean venous thromboembolism (VTE), pulmonary embolism (PE) and deep venous thrombosis (DVT) volume of PE patients with and without concomitant DVT

	All patients	**Patients with PE but without DVT (n=32)**	**Patients with PE and coexisting DVT (n=31)**
VTE volume (cm^3)	19.0 (13.0)	10.6 (8.3)	27.7 (11.1)
DVT volume (cm^3)	NA	NA	14.2 (11.8)
Proximal PE volume if present (cm^3)	6.1 (4.1)	5.4 (4.6)	6.8 (3.4)
Peripheral PE volume if present (cm^3)	5.9 (3.7)	5.2 (4.0)	6.7 (3.3)

Mean (standard deviation)
NA = Not available

9.1.4. Correlation of haemostatic markers with venous thromboembolism volume

We found that the acute phase FV PL as well as the acute phase platelet count correlated negatively with VTE volume. The acute phase FXIIIa also had a negative correlation with VTE volume. Additionally, acute phase D-dimer correlated positively with VTE volume (0.45, p<0.001). (Table 10)

Table 10. Correlation between the plasma levels of haemostatic markers in the acute and stable phases with venous thromboembolism (VTE) volume

The correlation of haemostatic markers with VTE volume is presented in Table 10.

Haemostatic markers	**Pearson correlation between the acute phase value and VTE volume**	***p*-value**	**Pearson correlation between 7-month value and VTE volume**	***p*-value**
Factor XIIIa (%)	0.23	0.07	−0.23	0.07
von Willebrand factor antigen (IU/ml)	−0.01	0.9	−0.05	0.7
Factor VIII (%)	−0.04	0.8	−0.07	0.6
Factor V (%)	−0.27	0.04	−0.12	0.3
Soluble thrombomodulin (ng/ml)	−0.08	0.5	0.22	0.08
Platelet count (E9/l)	−0.36	0.01	−1.4	0.5

9.1.5 Association of venous thromboembolism volume with patient characteristics and preceding medication

Fifteen out of the 63 patients were on antiplatelet medication when PE was diagnosed (Table 11). The preceding use of antiplatelet drugs (acetylsalicylic acid (ASA) or clopidogrel) had a significant association with VTE volume. The duration of PE symptoms did not show a significant association with VTE or PE volume. The correlation of VTE volume with age or BMI was not significant (0.14, *p*=0.3 for age, and 0.06, *p*=0.7 for BMI). Only 5 patients were diagnosed with the heterozygous FV variant, and VTE volume did not significantly differ from non-carriers.

Table 11. Association of venous thromboembolism (VTE) volume with specific patient characteristics

	Mean VTE volume (cm^3) (n)	***p*-value for the difference**
Gender (male/female)	21.2 vs 17.0 (30/33)	0.09
Current smoking (yes/no)	17.1 vs 19.4 (11/52)	0.6
Females with HRT or OC (yes/no)	14.1 vs 19.6 (15/18)	0.2
Previous DVT (yes/no)	21.3 vs 18.7 (7/56)	0.6
Duration of PE symptoms (≤1 week or >1 week)	20.9 vs 14.7 (44/19)	0.08
Preceding antiplatelet medication (yes/no)	12.3 vs 21.1 (15/48)	0.02
Thrombophilia (a heterozygous factor V variant) (yes/no)	25.4 vs 18.7 (5/57)	0.3

DVT = Deep venous thrombosis
HRT = Hormone replacement therapy
OC = Oral contraception
PE = Pulmonary embolism

9.1.6 Association of pulmonary embolism location and coexisting deep venous thrombosis

In 53 patients the PE was located both in proximal (pulmonary truncus, main pulmonary or lobar pulmonary) and peripheral (segmental or subsegmental pulmonary) arteries (Table 12). In 10 patients the PE was exclusively peripheral. None of the patients with exclusively peripheral PE had coexisting DVT ($p<0.001$). Noteworthy is the thorough exclusion of pulmonary illnesses from all patient which, which might othervise explain the exclusively peripheral PE location.

Table 12. Association of pulmonary embolism (PE) location with coexisting deep venous thrombosis (DVT)

	Coexisting DVT	
	Yes	No
Proximal and peripheral PE	31	22
Exclusively peripheral PE	0	10
$p<0.001$ for the difference		

9.1.7 Haemostatic markers and other characteristics in pulmonary embolism patients with and without coexisting deep venous thrombosis

The PLs of haemostatic markers measured either in the acute or stable phase did not differ between PE patients with and without coexisting DVT. The patients in these two groups were also otherwise similar in terms of patient characteristics. There were neither significant differences if only patients with exclusively peripheral PE were compared with patients with PE and DVT (Table 13).

Table 13. Patient characteristics and the plasma levels of haemostatic markers in the acute phase in pulmonary embolism (PE) patients with or without coexisting deep venous thrombosis (DVT)

	PE with coexisting DVT (n=31)	**PE without coexisting DVT (n=32)**	***p*-value**
Age (years)	57.3 (17.1)	55.7 (17.6)	0.7
Gender male/female	18/13	19/13	0.1
BMI (kg/m^2)	29.7 (5.4)	29.3 (5.6)	0.7
Preceding immobilization yes/no	13/18	19/13	0.2
Factor V (%)	106 (22.4)	115 (21.6)	0.1
Factor VIII (%)	165 (56.7)	170 (559)	0.7
Factor XIIIa (%)	82.4 (18.8)	92.5 (29.1)	0.1
von Willebrand factor antigen (IU/ml)	2.73 (1.44)	2.58 (0.97)	0.6
C-reactive protein	33.3 (39.1)	39.3 (29.4)	0.5
Fibrinogen (g/l)	4.4 (1.5)	4.1 (1.3)	0.4
Mean (standard deviation) BMI = body mass index			

9.2 Study IV and additional information

9.2.1 Pulmonary embolism mortality

During the analysed time period (1996–2017), in total 1,101,806 deaths occurred in Finland and 25,163 (2.3%) of these were defined as PE deaths (Table 14). Of these PE deaths, in 15,759 (62.6%), 3703 (14.7%) and 5701 (22.7%), PE was listed as the immediate, underlying and immediate, or a contributory cause of death, respectively. 59% of the subjects were female (14,738). Female subjects were older than male subjects (78.1 years vs 72.3 years, $p<0.001$).

PE mortality decreased by approximately 28% (AAPC −1.4%) during 1996–2017 and the change was more profound if only cases where PE was defined as the immediate cause of death were analysed, i.e. 55% (AAPC −3.0%) (Figure 9-10). The decrease in the number of PE deaths in females was twofold compared with males (for females, AAPC −2.1%, $p<0.001$, and for males, AAPC −1.0%, $p<0.001$) ($p<0.001$ for the difference). The change in age-adjusted PE mortality in the calculated standard population was even more significant at 52% (AAPC −3.2%, $p<0.001$).

Table 14. Characteristics of the subjects with pulmonary embolism (PE) as a cause of death

	Male	**Female**
PE deaths	10,425	14,738
Mean age (standard deviation)	72.3 (13.0)	78.1 (12.2)
Determination of PE diagnosis based on (%) autopsy clinical evaluation	 3931 (36.3) 6494 (63.2)	 6271 (41.8) 8467 (57.8)
PE determined as Immediate Underlying and immediate Contributory cause of death	 6620 (63.4) 1355 (13.0) 2450 (23.6)	 9139 (61.9) 2348 (16.0) 3251 (22.1)

Figure 9. Annual number of pulmonary embolism (PE) deaths in 1996–2017

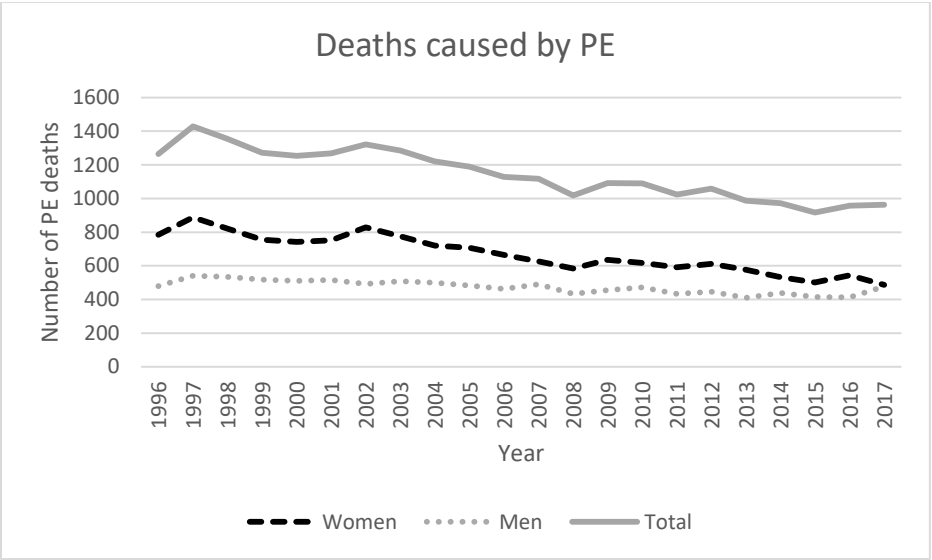
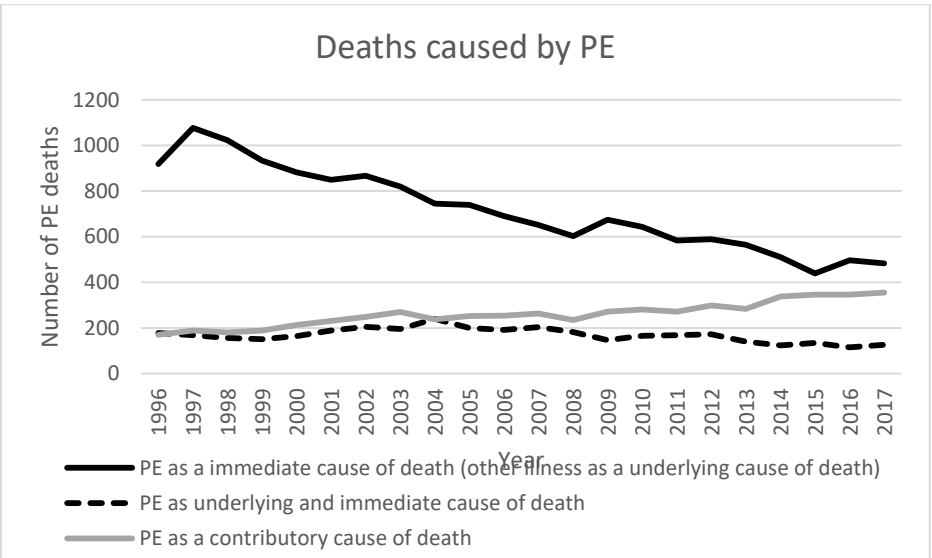


Figure 10. Annual number of pulmonary embolism (PE) deaths per 100,000 inhabitants in different causes of death categories



9.2.2 Autopsy rate and practice patterns of cause of death recording

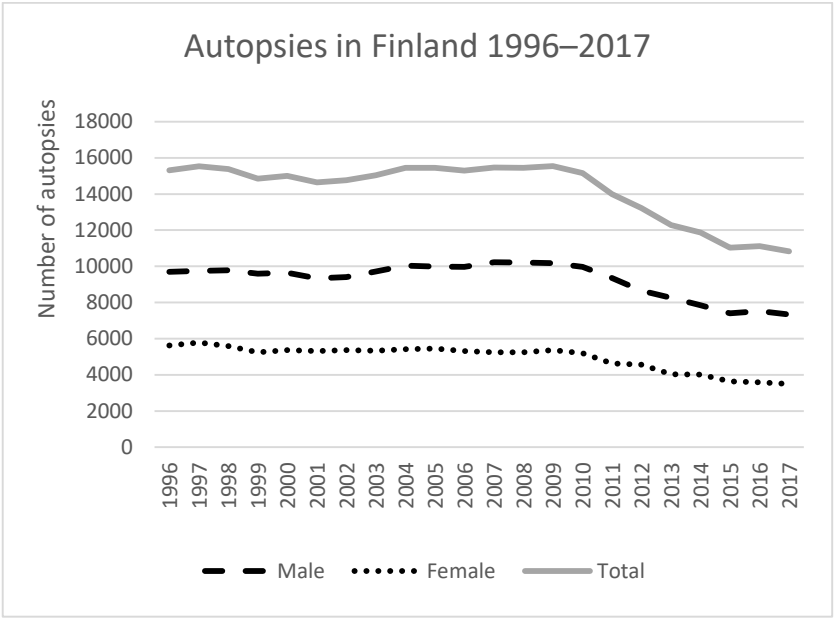
The yearly number of autopsies decreased during the examined time period, 1996–2017. However, the autopsy rate remained stable during 1996–2009 and the decline began as late as 2010 (Figure 11). In total, 4561 (30%) fewer autopsies were performed in 2017 than in 1996 and the number of autopsies and the proportion of autopsies performed relative to all deceased decreased during 1996–2017 (15,391/49,168, 31.3% in 1996, and 10,830/53,722, 20.2% in 2017, p>0.001). The annual change in the number of autopsies after 2009 is shown in Table 15. The number of PE deaths showed a significant association with the number of autopsies, and possibly 60 PE deaths could have been diagnosed if 1000 autopsies had been performed (p<0.001).

During the study period, an autopsy was performed in 60% (14,961/25,163) of the patients with PE death. The AAPC of PE mortality was relatively similar before and after 2010 when the autopsy rate started to decline (AAPC −1.4%, p<0.001, for years 1996–2009, and AAPC −1.4%, p=0.01, for years 2010–2017) (p=0.72 for the difference).

Table 15. Annual decrease in number of autopsies, incidence of pulmonary embolism (PE) deaths in autopsy and estimated PE mortality

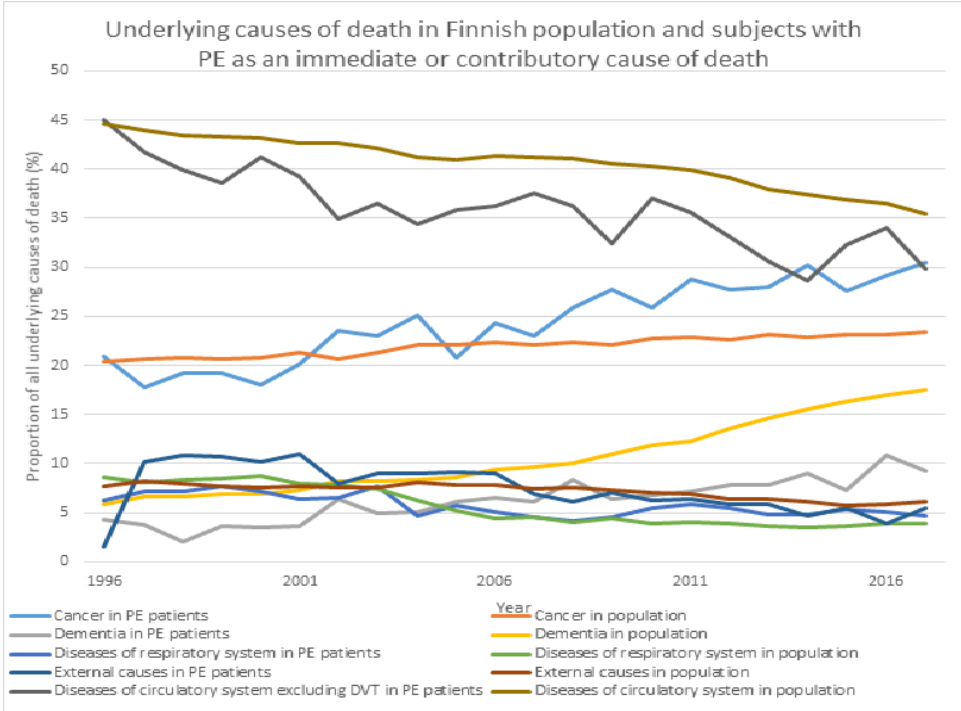
Year	Decrease in number of autopsies compared with 2009	Incidence of PEs in autopsy relative to all autopsies (%)	Number of possibly missed PEs due to the decrease in the number of autopsies compared with 2009 (all diagnosed PE deaths)	Estimated PE mortality if possibly missed PE deaths are taken into account (per 100,000 inhabitants)
2010	−290	4.3	12 (1090)	20.0
2011	−1463	4.2	58 (1024)	19.8
2012	−2232	4.4	90 (1058)	20.9
2013	−3171	4.3	126 (987)	20.4
2014	−3583	4.4	143 (972)	20.3
2015	−4419	4.3	176 (917)	19.9
2016	−4342	4.5	173 (957)	20.4
2017	−4624	4.6	185 (964)	20.5

Figure 11. Annual number of autopsies at population level



Both in the Finnish population as a whole and in the subpopulation with PE as the immediate or a contributory cause of death, the most common illness categories defined as the underlying cause of death were diseases of the circulatory system (48.9%), neoplasms (21.2%), external causes (9.3%), dementia (5.5%) and diseases of the respiratory system (4.2%). The proportion of these illnesses as the underlying cause of death and the change in the proportion of these illnesses in PE patients and in the whole population were similar except for dementia, the proportion of which approximately doubled in PE patients (4.2% in 1996 and 7.8% in 2017) but tripled in the whole population (5.9% in 1996 and 17.8% in 2017, $p<0.001$). Also, cancer became a more common underlying cause of death in PE patients than in the whole population (20% both in the whole population and in PE patients in 1996, and 23.4% in the whole population and 29.8% in PE patients in 2017, $p<0.001$) (Figure 12).

Figure 12. Proportion of most common categories for underlying cause of death in the population and in analysed subjects with pulmonary embolism (PE) as the immediate or a contributory cause of death (DVT = deep venous thrombosis)



9.2.3 Pulmonary embolism incidence in Finland (additional information)

In total, 100,537 patients with PE as the discharge diagnosis during 1997–2017 were identified. During follow-up, the yearly incidence more than doubled from 42/100,000 in 1997 to 103/100,000 in 2017, and in 2017 approximately 5700 patients had PE as their discharge diagnosis (Figure 13). 57% of the patients were female. PE incidence increased substantially with age, being 6.2, 46.8, 194.2 and 419.9 per 100,000 inhabitants in 2017 for the following age quartiles: 0–24, 25–49, 50–74 and >75 years, respectively. The increase in PE incidence was highest in younger age quartiles between 1996 and 2017 (Table 16).

Figure 13. Incidence of pulmonary embolism (PE) in Finland per 100,000 inhabitants

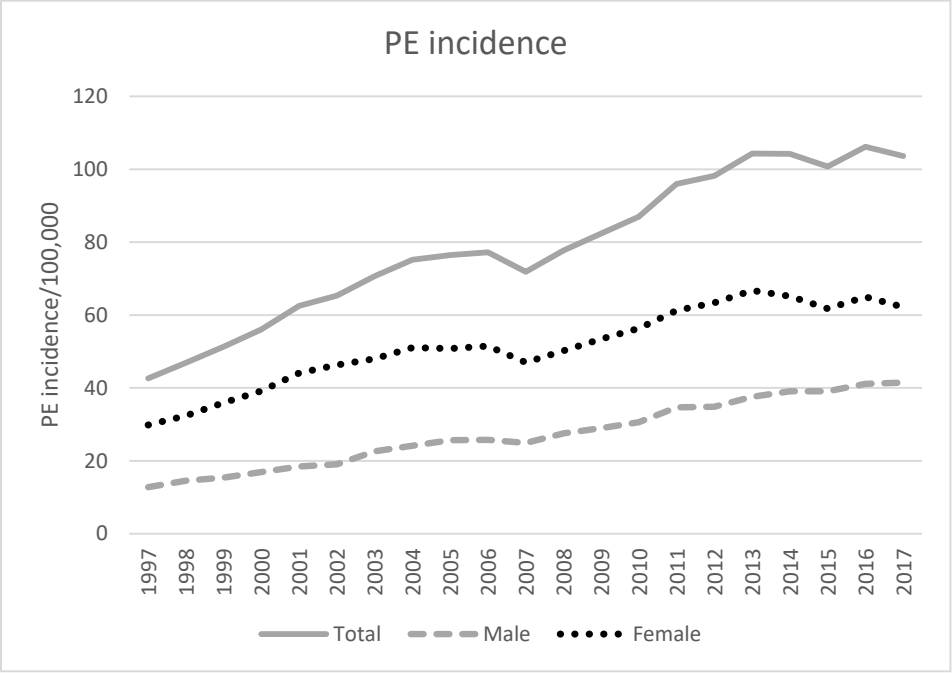


Table 16. Annual pulmonary embolism (PE) incidence per 100,000 inhabitants in Finland in gender-divided age quartiles

	PE incidence/100,000 in age quartiles			
Year	**0–24 years (male/female)**	**25–49 years (male/female)**	**50–74 years (male/female)**	**>75 years (male/female)**
1997	0.9 (0.3/1.5)	11.8 (12.9/10.7)	88.8 (83.7/93.4)	337.4 (308.9/349.8)
1998	2.3 (0.7/4.0)	15.0 (14.1/15.8)	97.2 (92.7/101.3)	336.4 (325.9/340.9)
1999	2.6 (1.4/3.8)	18.9 (16.8/21.2)	100.0 (92.0/107.3)	360.3 (338.1/370.2)
2000	2.1 (0.9/3.3)	20.4 (18.3/22.6)	107.0 (101.0/112.5)	403.9 (389.5/410.4)
2001	3.5 (1.2/6.0)	23.7 (22.8/24.6)	112.3 (103.8/120.2)	448.1 (406.7/467.3)
2002	3.6 (2.2/5.1)	26.6 (24.3/28.9)	114.0 (102.7/124.6)	446.7 (384.1/476.6)
2003	4.6 (1.1/8.3)	31.5 (31.9/31.1)	126.0 (120.1/131.6)	448.4 (418.8/462.8)
2004	4.7 (1.5/8.2)	30.9 (27.1/34.8)	134.3 (134.6/134.0)	461.8 (402.5/491.4)
2005	6.2 (2.0/10.7)	35.5 (32.4/38.7)	132.9 (136.2/129.8)	458.3 (440.4/467.3)
2006	5.5 (2.2/9.0)	37.7 (37.0/38.5)	131.1 (129.8/132.2)	446.6 (403.7/468.9)
2007	4.5 (2.3/6.7)	34.6 (31.1/38.1)	122.9 (129.1/117.1)	413.4 (390.4/425.6)
2008	4.9 (2.6/7.3)	38.5 (35.9/41.2)	135.6 (139.9/131.5)	421.0 (403.6/430.3)
2009	5.8 (2.9/8.9)	41.0 (37.2/45.0)	141.5 (146.8/136.5)	441.6 (415/455)
2010	6.3 (3.7/8.9)	41.5 (38.3/44.8)	151.0 (153/148.4)	461.0 (445/469.9)
2011	6.1 (3.4/8.9)	45.8 (41.8/50.1)	169.9 (177.0/163.3)	476.4 (440.2/497.0)
2012	7.1 (3.7/10.6)	47.5 (41.9/53.3)	173.4 (176.1/170.9)	480.0 (456.5/493.6)
2013	7.3 (3.5/11.3)	49.6 (44.5/55.0)	185.7 (191.1/180.7)	483.4 (434.7/512.2)
2014	7.4 (4.1/10.8)	47.9 (44.0/52.1)	189.3 (198.9/180.1)	479.9 (470.0/485.8)
2015	7.1 (3.6/10.7)	46.8 (44.5/49.1)	188.9 (197.8/180.4)	437.3 (456.4/425.6)
2016	6.4 (4.2/8.8)	49.0 (45.1/53.1)	199.6 (209.7/189.8)	431.1 (415.2/441.0)
2017	6.2 (3.8/8.6)	46.8 (47.3/46.1)	194.2 (208.3/180.8)	419.8 (400.2/432.2)

9.3 Summary of results

1. The PL of FXIIIa was lower, whereas the PLs of vWF:Ag, Fg, D-dimer and trendwise also FVIII were higher on admission compared with in the stable phase. In addition, in the stable phase, PE patients had higher vWF:Ag levels compared with healthy controls, but levels of FXIIIa did not differ between the groups.
2. The volume of VTE correlated inversely with FV and platelet count, and positively with D-dimer levels measured in the acute phase, and the preceding use of ASA or clopidogrel was associated with smaller VTE volume.
3. Patient characteristics or the PLs of haemostatic markers did not differ between PE patients with or without coexisting DVT. However, the location of the PE was associated with DVT coexistence as none of the patients with exclusively peripheral PE had coexisting DVT and the PE was proximal in every patient who had coexisting DVT.
4. PE mortality decreased by 28% during 1996–2017 in Finland. A significant association between the annual number of autopsies and PE deaths was seen and when adjusted for the changes in autopsies indicates that after 2009 PE mortality plateaued.

10. Discussion

10.1 Main findings

Here is reported for the first time the long-term PE mortality trend in Finland. This thesis also provides data on how the previously disregarded autopsy rate at the population level influenced the PE mortality trend. PE mortality decreased by approximately 1.4% annually during 1996–2017. It decreased despite the autopsy rate remaining unchanged until 2010 and the trend did not accelerate, although the autopsy rate decreased rapidly afterwards.

10.2 Plasma levels of haemostatic markers in acute pulmonary embolism and 7 months later (study I)

The events of the coagulation system during thrombus formation are well known at the cellular level but relatively few studies have evaluated the time-related changes of individual coagulation factors in the evolving *in vivo* environment of

acute PE. The results in our study verify the well-known dynamics of D-dimer during acute VTE. All patients had an elevated PL of D-dimer in the acute phase.
During follow-up, the D-dimer level decreased but remained over the upper limit of the reference value in 13 (20.6%) patients 7 months after diagnosis. PL of Fibrinogen, a known acute phase reactant, was temporarily elevated during acute PE, but there was no difference in stable phase Fg between PE patients and healthy controls.

vWF levels were also elevated during acute PE. Although they decreased during follow-up they were still significantly higher at 7 months compared with healthy controls. vWF has an important role in haemostasis and in thrombus formation (chapter 6.1.1) (Brill 2011 Chauhan 2007). Its deficiency or dysfunction cause a serious bleeding disorder (Nummi 2019), whereas high levels of vWF have been linked to an increased risk of VTE (Rietveld et al. 2019, Edvardsen et al. 2021).

vWF is secreted from platelets and the endothelium during the coagulation process but small amounts of it are constantly circulating in plasma (Mannucci 1998). The quantity of plasma vWF is measured by a vWF antigen test (vWF:Ag) and its functionality by various vWF activity tests. The PL of vWF:Ag has been evaluated in one study with 77 patients with either DVT or DVT and PE and it was seen that the vWF:Ag level was higher in the acute phase than in normal volunteers (Yamada et al. 1995). The present results are in line with this previous report. However, we expanded the understanding of vWF changes in acute thrombosis by showing that although at a stable phase the vWF:Ag was decreased, the level was still higher than in healthy controls. This finding is in line with the theory and previous case control study reporting that a high vWF:Ag level increases the risk of VTE (Rietveld et al. 2019).

vWF and FVIII circulate in complex – vWF serves as a carrier protein for FVIII and protects it from inactivation (Franchini and Lippi 2006). Similarly to vWF, also FVIII is a non-specific acute phase reactant. Tichelaar et al. evaluated FVIII in 75 patients with either DVT or PE by measuring its PL in the acute phase and twice during a follow-up of approximately 6 months. It was seen that almost 90% of the patients had elevated values in the acute phase compared with the reference value and that the FVIII level decreased from the acute phase during follow-up (Tichelaar et al. 2012). This is in line with our results, although the change in FVIII level was not statistically significant in our cohort. Similarly to vWF, the elevated FVIII has also been attributed as a risk factor for VTE (Rietveld et al. 2019). Interestingly, in our data the mean FVIII PL in the stable phase was higher than the upper range of normal variation in 38% (24/63) of the patients.

Activated FXIII is attached to the fibrin network and cross links the fibrin chains, thus stabilizing the forming thrombus (Figure 1). There is evidence that acute VTE is associated with a consumption-based transient decrease in FXIII levels in circulating blood, and in a murine model it was shown that a 50% reduction in plasma FXIII concentration produced significantly smaller venous thrombi (Kattula et al. 2018). *In vivo*, two studies have evaluated the FXIII PL in 71 and 41 PE patients, and compared the results with patients in whom PE was excluded. The results were similar – the FXIIIa level was lower in PE patients than in patients without PE and other final diagnoses such as acute coronary syndrome, spontaneous pneumothorax or respiratory disease (Kucher et al. 2003, Tang et al. 2017). We also showed that the FXIIIa level was lower in the acute phase than in the stable phase in PE patients and that the stable phase values were similar with healthy controls. According to available data, FXIII levels/activity are not associated with VTE recurrence (Ząbczyk et al. 2021).

As described earlier (chapter 6.5.5), PE diagnostics faces a dilemma as the use of imaging studies has increased markedly but the yield of these studies has decreased simultaneously. The specificity of D-dimer is poor and consequently new modifications to diagnostic algorithms which mainly concentrate on increasing the D-dimer threshold for referral studies have emerged (Righini et al. 2014, van der Hulle et al. 2017, Kearon et al. 2019). It has been suggested that PE diagnostics might be improved by utilizing FXIII assessment in connection with D-dimer (Tang et al. 2017), but so far no clinical implications exist where D-dimer is analysed with FXIIa or other haemostatic markers.

10.3 Manifestation of venous thromboembolism (studies II–III)

We investigated how the presence or absence of concomitant DVT and the combined thrombus volume of PE and DVT were associated with haemostatic markers and patient characteristics.

10.3.1 Differences between pulmonary embolism patients with or without coexisting deep venous thrombosis

The manifestation of the VTE can vary from distal DVT to proximal PE and these also commonly coexist. It is widely assumed that PE is a sequel of DVT (Kearon 2003) but this view has been challenged as in many cases coexisting DVT is not found in PE patients. In our patient cohort only half of the PE patients had detectable DVT in ultrasound, which is in line with previously available data.

It has been argued that an explanation for this would be that the ultrasound examination was insufficient as only proximal veins were evaluated in older studies (Palareti et al. 2019). However, in our data, calf veins were also investigated and in a study that used a total-body magnetic resonance direct thrombus imaging technique for thrombus search, the origin of the PE was not evident in half of the cases.

We found, interestingly, that DVT coexistence was clearly associated with PE location as none of the patients with exclusively peripheral PE had coexisting DVT and the PE was proximal in every patient who had coexisting DVT. Similar results were also seen in a study with 141 PE patients where the prevalence of DVT was nearly twofold in subjects with proximal versus peripheral PE (66.8% vs 36.4%) (Lee et al. 2016).

Manifestation of VTE (PE or DVT) has been shown to be associated with short-term prognosis and recurrence rate. PE patients with coexisting DVT have a 1.9-fold higher risk of short-term mortality and a 1.1- to 1.3-fold risk of fatal PE recurrence than isolated PE or isolated DVT patients (Becattini et al. 2016, Douketis et al. 2007). The explanation for this difference is not clear. Theoretically, coexisting DVT could continuously embolize and therefore increase the PE volume but on the other hand the use of vena cava filters has not been shown to improve short-term prognosis (Konstantinides et al. 2020). The current guidelines do not recommend the exclusion of DVT from all PE patients and the existence of DVT is not incorporated in the indexes that evaluate the risk of short-term mortality in clinical practice (Aujesky et al. 2005, Jiménez et al. 2010). The absolute difference in 30-day mortality between patients with PE with or without coexisting DVT is 2.4% (relative risk 63%) (3.8% for PE and 6.2% for PE+DVT). Although this difference is not negligible, further studies are needed to evaluate whether this difference can be affected by increasing the intensity of hospital care or anticoagulation (Becattini et al. 2016).

10.3.2 Venous thromboembolism volume (study II)

VTE volume has clinical and prognostic significance. For clinical purposes, a very rough description of volume (proximal, distal, massive, non-massive) is sufficient. A need for more accurate VTE volume assessment for research purposes is obvious. However, more accurate quantification of total VTE volume of individual patients is problematic. Previously existing semiquantitative indexes for PE and DVT are not comparable. Previous studies showing a correlation of D-dimer level with thrombus volume have focused either only on PE or DVT (Ghanima et al. 2007, Kurklinsky et al. 2011), and a lack of inclusion of the combination of PE and DVT (total VTE volume) has been a weakness.

In this study, we wanted to approximate the volume of both PE and DVT in a commensurate way and to combine these, and we developed a semiquantitative estimate of the total VTE volume from the imaging used in clinical practice.
To our knowledge this is the first study which has used the estimation of total VTE volume in this context. The total VTE volume estimation used was based on the identification of thrombotic segments of vasculature and the known length and diameter of the corresponding veins and pulmonary arteries, and it had a strong positive correlation with D-dimer. The range of VTE volume in our PE patient cohort was notable (0–57 cm^3). Patient gender, BMI, symptom duration or various risk factors for VTE were not associated with total VTE volume.

From the coagulation markers assessed, we found that acute phase FV and platelet count showed a modest but significant correlation with total VTE volume.

FV together with FX and Ca+ (Figure 1) form a prothrombinase complex on the negatively charged phospholipid surfaces. The prothrombinase complex produces most of the thrombin during the propagation phase (Hoffman and Monroe 2001). Besides circulating plasma FV, FV released from platelet α-granules upon their activation (Monroe et al. 2002) is available during clot formation. We found that a significant negative correlation between acute phase plasma FV and VTE volume might be due to the consumption of FV – the larger the thrombus the more plasma FV is bound into the forming clot. To our knowledge, no previous studies have reported the behaviour of FV levels in comparison with VTE *in vivo*, neither are we aware of any experimental studies evaluating the consumption of FV in relation to venous thrombus volume. Indirect evidence of the role of FV in venous thrombosis comes from the FV Leiden mutation, which prevents FV deactivation and is associated with an increased risk of VTE. To our knowledge, no studies assessing the association of VTE volume and FV Leiden mutation in humans exist. Available data from a murine model show that venous thrombi induced by electrical injury were two times larger in homozygous FV mice versus wild-type mice (Cooley et al. 2005). In our cohort, VTE volume was similar in patients with or without FV Leiden mutation but the FV Leiden subgroup was too small (n=5) to draw any conclusions.

We found that thrombus volume was smaller in patients (n=15) who were on antiplatelet medication (ASA or clopidogrel) at the onset of PE. Only less than 1% of the total volume of PE consists of platelets (Chernysh et al. 2020), but the important role of the negatively charged phospholipid surface of activated, clot-bound platelets in tenase and prothrombinase complex formation is obvious. Our finding is interesting since the established literature has confirmed the effect of platelet inhibitors in VTE prophylaxis (PEP Collaboration 2000, Weitz et al A. 2017, Anderson et al. 2018). In the landmark Pulmonary Embolism Prevention Trial, ASA was shown to reduce the risk of fatal PE by 58% compared with placebo (PEP Collaboration 2000) and was seen to be as efficient as rivaroxaban in preventing

VTE after knee or hip arthroplasty (Anderson et al. 2018). However, no previous data on the direct effect of platelet inhibitors on VTE volume are available according to our knowledge.
Since VTE volume has prognostic significance, this unique finding, although in a very small patient population, is interesting and worthy of further research.

The platelet count in the acute phase correlated negatively with VTE volume. Unfortunately, we did not have information on the patient baseline level of platelet count available and thus the analysis of platelet consumption and platelet count recovery was incomplete.

10.4 Pulmonary embolism incidence

PE incidence in high-income countries has grown in recent years (Munster et al. 2019, Kempny et al. 2019, Keller et al. 2020, Wiener et al. 2011) but until now data from Finland have been lacking. We have now shown that the trend and incidence in Finland are similar to other Western countries (chapter 9.2.3). This increase is a sum of many factors. Firstly, people live longer and other risk factors for PE have become more common. The increased incidence has also been attributed to more accurate diagnostics leading to the detection of smaller and even asymptomatic cases. The observation from the USA of PE incidence more than doubling but PE mortality decreasing by only 3% from 1998 to 2008 aroused suspicion regarding diagnostics (Wiener et al. 2011). It is noteworthy that the proportion of haemodynamically unstable patients relative to all patients with confirmed PE diagnosis has remained the same despite PE incidence markedly increasing (Keller et al. 2020). This indicates that the increased diagnostics of insignificant small PEs does not entirely explain the markedly increased PE incidence, as otherwise the proportion of haemodynamically unstable patients should have decreased. Additionally, preliminary data from Helsinki and Uusimaa Health Care District have shown that despite the use of CTPA increasing by 30% in 2014–2018 the PE incidence plateaued at the same time (Sane unpublished results).

10.5 Pulmonary embolism mortality in Finland (study IV)

The analysis of PE mortality trends in the population is important as it provides real-world information on the burden of PE. In addition, the efficacy and the need for further advances in PE management can be estimated as improvements made in prevention and treatment should eventually lead to decreased PE mortality. Our results show that PE mortality has decreased by nearly 28% and age-adjusted mortality by 52% in the past 20 years.

The method used in our study was, regarding the collection of subjects with PE death, similar to that used in previous corresponding studies in other developed countries (Shiraev et al. 2013, Wiener et al. 2011, Olié et al. 2015, Barco et al. 2020(B), Martin et al. 2020).

However, these previous studies differed in terms of what type of PE deaths were analysed, i.e. PE as an immediate, underlying or contributory cause of death, and whether the types of PE deaths were all or partially included. This understandably affects the incidence results and observed changes in PE mortality as demonstrated in Table 4 and underlines the importance of considering the definition of PE death in each study when interpreting the results. In addition, the customs surrounding the completion of death certificates likely vary from country to country and this should be kept in mind when PE mortality statistics are interpreted. For example, in a recent study focusing on PE mortality in 41 European nations (Barco et al. 2020(B)) in which only cases where PE or DVT was defined as the underlying cause of death were analysed, over 10-fold differences in the incidence of age-adjusted PE mortality per 100,000 inhabitants between countries was seen. For example, Bulgaria, North Macedonia and Finland were reported to have 24.0, 1.2 and 5.3 PE deaths per 100,000 inhabitants, respectively, which is unlikely to be caused by differences in PE management. In our data, only a minority (15%) of patients had PE defined as the underlying cause of death and it is clear that the results can only be explained by variation between the countries in how PE death is classified.

Advanced diagnostics might nowadays improve the detection of the root cause of PE and therefore PE mortality might be underestimated if only cases where PE was the underlying cause of death are evaluated. The exclusion of cases where PE is defined as a contributory cause of death is also problematic as when the burden of PE to society is evaluated also the cases where PE has had an adverse effect on general health leading to death are important to include. To avoid the risk of underestimation of PE mortality we concluded that when evaluating the mortality trend of a specific illness, the inclusion of all three causes of death classes is justifiable. However, we reported the results also separately according to cause of death classification. A similar method was also used in a recent study from the USA (Martin et al. 2020).

Unlike previous studies, we also reported the simultaneous changes in autopsy rate and we showed that the numbers of autopsies and PE deaths were significantly associated. It was evaluated that 60 PE deaths could be diagnosed per 1000 autopsies performed. Thorough medicolegal investigation leads to a reliable definition of the cause of death, but autopsy policy and customs in Western countries have changed substantially during recent decades. At present, a higher uncertainty concerning the cause of death is accepted.

From 1980 onward, autopsy rates have declined in most Western countries, although it is well known that a higher autopsy rate guarantees the quality of medical care (WHO 2015, Friberg et al. 2019).

Considering the challenges of ante-mortem diagnostics of PE, PE deaths can be missed if autopsies are not performed. In our data, the PE incidence of all autopsies was nearly 5%, which is in line with the previous results when the data from a single academic medical centre were evaluated (Sweet et al. 2013). Noteworthy in Finland is that the decrease in the autopsy rate began much later when compared with the USA, where autopsy data are available from the publication of Hoyert. In 2010 the autopsy rate was almost four times higher in Finland than in the USA (30% vs 8.5%) (Hoyert 2011). In the USA, the autopsy rate has been low (8.5%) since the beginning of the 1980s, questioning the reliability of the comparison of PE mortality statistics with countries adhering to different contemporary autopsy policy.

Fortunately, during the first 13 years of our study period, in contrast to other Western countries, the autopsy rate in Finland remained unchanged and, in an international context, at a fairly high level. During this period, 1996–2010, significant advancements in PE diagnostics, treatment and prevention were achieved. Unique Finnish data from these years allowed us to analyse the trends in PE mortality data without the confounding effect of a changing autopsy policy rate. Therefore, we could confirm an autopsy-independent decrease in PE mortality during 1996–2009, which is likely caused by an improvement in PE management (Bikdeli et al. 2020). After 2010, the autopsy rate started to decline also in Finland, and the decline continued constantly by approximately 5% annually. Therefore, the mortality data afterwards should be interpreted carefully. We do not know what the PE mortality trends after 2010 in Finland would look like without the decrease in the autopsy rate. If estimated by taking into account the PE incidence of all autopsies and the annual decrease in the number of autopsies, the PE mortality would have plateaued after 2009. A similar finding was reported from the USA where the autopsy rate has remained at a low and stable level so far this century. There it was seen that after 2008 the PE mortality actually increased by 0.6% annually.

In the 2010s, no radical advances affecting the prognosis of PE were made. It is possible that earlier advances made in PE treatment and diagnostics have been compromised by the increased PE incidence. Our results highlight the need to further develop PE management for better prognosis.

10.6 Overall study strengths and weaknesses

10.6.1 Studies I–III

In studies I–III we analysed the PL of a wide variety of haemostatic markers of a relatively large sample of thoroughly – both clinically and by imaging – investigated patients and healthy controls. The patient cohort characteristics were in line with the typical characteristics of PE patients in other larger cohort studies.

Control of artificial variability in haemostatic markers vulnerable to errors in medication and other pre-analytical factors in a clinical setting is crucial but challenging. In order to minimize the errors, the study protocol was carefully planned beforehand. A weakness is that several interesting and informative coagulation markers could not be included in the protocol because of the interference of unavoidable anticoagulation treatment in a real-life patient population. On the other hand, data from real-life PE patients are needed to explore and understand the time-dependent changes effected by a complex interplay of plasma and tissue components of the coagulation system *in vivo*. The investigated markers were chosen so that they are undisturbed by anticoagulation treatment. Special attention was paid to sample collection, handling and storage, etc., guided by a detailed written protocol. Analysis of the samples was undertaken in national reference laboratories.

Values of coagulation markers have significant interindividual variability, and each subject served as his or her own control, which made an ideal study setting to evaluate the true changes in PLs of haemostatic markers over time possible.

The age- and gender-matched control group allowed a parallel comparison when analysing the PLs of haemostatic factors in the stable phase in PE patients.

The study group of 63 PE patients could be argued to be small, but it is considerably large when account is taken of the inclusion of a combination of thorough analysis of haemostatic parameters, radiological imaging, methods of clinical physiology and repeated measurements during follow-up.

Interpretation of the CT scans and calculation of the Mastora score were undertaken by two expert radiologists blinded to each other's results. The possible interobserver disagreement was discussed and eventually all the Mastora scores were reported in agreement.

The idea to combine the thrombus volume of both DVT and PE was new and not validated. However, the calculation of DVT volume (Ouriel 1999) was previously reported, the logic behind the method is simple and the basic arithmetic used in the volume calculation is solid. The method for PE volume evaluation (Furlan et al. 2011) has been previously well studied and in our opinion gives a reliable estimation of PE volume. The strong correlation of D-dimer with our VTE volume estimation also supports this methodology. Overall, the study setting can be considered to be valid and to enable the evaluation of the prespecified study questions.

10.6.2 Study IV

The use of registry data to evaluate PE mortality is the best option to analyse the trends of nationwide changes of certain illnesses. However, awareness of the strengths and weaknesses of Finnish registries and demographic data is important.

Firstly, Finland has universal healthcare run mainly by municipalities and controlled by the state. This ensures that the data truly describe the nationwide statistics. Secondly, the practice patterns in the cause of death determination in Finland increase the reliability of mortality statistics. The death certificates in Finland are checked for correctness by a forensic pathologist. The autopsy rate is higher than in other European Union member states (WHO 2015, and compared with the USA the autopsy rate was almost four times higher in 2010. Post-mortem examination with autopsy is mandatory if a medical or surgical operation is performed before the death, which ensures that in most patients with a strong risk factor for PE before their death, the PE is definitely included. In addition, a third-party study has stated that the quality of civil registration and vital statistics including death statistics is very high in Finland (Mikkelsen et al. 2015), and when compared with Denmark, ill-defined and unknown causes of death in death certificates is 13 times less frequent (Ylijoki-Sørensen et al. 2014). A weakness is that the correctness of PE diagnosis in the cause of death registry in Finland has not been validated. In the Swedish National Patient Registry and Cause of Death Registry it has been shown that PE diagnosis had a high positive predictive value of up to 86%, indicating that the registry data regarding PE are reliable (Öhman et al. 2018).

Thirdly, the population demographics have changed substantially in Finland in the past 20 years due to ageing of the population. Ageing has a significant impact on PE incidence and mortality. We used the registry data of population-based demographic changes in Finland including age distribution for each 5-year period, which are available from the StatFin open database. This enabled the calculation of age-adjusted results and ensured that the impact of demographic changes was taken into account in the analysis of PE mortality statistics.

The most significant weakness in our data as well as in other studies analysing PE mortality is the lack of a standardized definition for PE death (Tristchel 2020, leading to its variable notation in the register by clinicians since PE's role in the chain of events leading to death can be diversely interpreted. This may cause variation in reporting PE as a cause of death. As we analysed the trend for all PE deaths we believe that the impact of the lack of an unambiguous definition of PE death was similar during the whole study period and therefore did not cause a marked distortion in the trend analysis.

Although the national guidelines and legislation for defining the cause of death remained unchanged during the follow-up period, the practice patterns can also change due to changes in the surrounding society.

It is possible that nowadays the specific cause of death may be less often defined especially for old multimorbid patients and this may explain why the number of autopsies has decreased especially in older subjects. The usefulness of determining the specific cause of death for old multimorbid patients can be challenged but it is important to note that changes in these customs may also be reflected in the mortality statistics of specific illnesses.

11. Summary and conclusions

The results of studies I–IV can be summarized in the following conclusions.

I. There were acute PE-induced changes in the PLs of several haemostatic markers when compared with the stable phase 7 months later. The most significant differences between the acute and stable phases were noticed in D-dimer, FXIIIa and vWF:Ag. We also noticed that the stable phase values of vWF:Ag in PE patients differed from healthy controls.

II. The total combined volume of VTE can be estimated from clinical imaging by using semiquantitative evaluation of pulmonary artery obstruction from CTPA, anatomical data on the location and extent of DVT by ultrasound, and the average volumes of vein segments in humans reported in the literature. We showed that the total volume of VTE had marked variation in the PE population. The total VTE volume correlated very strongly with D-dimer and negatively with the acute phase FV PL and platelet count. Additionally, VTE volume was smaller in patients with preceding antiplatelet medication.

III. Coexisting DVT examined by bilateral leg ultrasound was found in 31 of 63 PEs. The coexistence of DVT was associated with a more proximal location of PE: all patients with DVT had proximal PE, and none of the patients with exclusively peripheral PE had coexisting DVT. The patient characteristics and PLs of haemostatic markers did not differ between PE patients with or without coexisting DVT.

IV. PE mortality decreased by 28% during 1996–2017 in Finland. PE mortality decreased also during the early study period of 1996–2009 when the autopsy rate was unchanged and at a high level (30%) in an international context, indicating that the decrease is 'real'. The decrease in PE death continued when the autopsy rate started to decline rapidly after 2010 but this can be partially caused by an underestimation of PE deaths due to less active determination of the specific cause of death.

12. Acknowledgements

This thesis was conducted in Central Finland Central Hospital in 2016–2018 and in Helsinki University Hospital Heart and Lung Center in 2019–2021. I am grateful for being able to work in these two inspiring hospitals. On the way to becoming a cardiologist numerous people have enabled me to work on this project. A few people deserve a special mention.

Firstly, I am thankful to my supervisor, Docent Pirjo Mustonen, who had originally been involved with this project and offered me the opportunity to deepen my understanding of PE by analysing the data that were buried in her office. Pirjo has been more inspiring and encouraging than I could ever hope a supervisor to be. The sessions in which we drafted the articles together were extremely educational and I can only admire Pirjo's patience towards my impatience.

Secondly, I am grateful to my other supervisor, Professor Jari Laukkanen, who has helped me during this whole process and always commented on my drafts on time with good insight into how to improve the text. In addition, Jari was helpful in clinical settings when I started my career as a cardiologist in Central Finland Central Hospital.

Thirdly, I want to thank Veli-Pekka Harjola, Marit Granér, Anneli Piilonen, Merja Raade, Miia Laiho and Päivi Piirilä, who originally were involved with the planning and carrying out of the project that was the foundation of this thesis. In a sports term, their work prepared me for an open goal which I could not miss. I also want to thank Professor Reijo Sund for valuable tips for improving the fourth publication, on PE mortality.

I would like to thank Professor Ari Palomäki and Docent Anu Kuittinen for finding the time to be the official supervisors and reviewing this thesis.

In addition, I want to thank all my friends and colleagues who have helped me on the way and offered a good balance between work and leisure. In particular, I want to thank my friend from medical school, Aleksi Reito, for hands-on training in SPSS and valuable guidance in the world of statistics, and Vuokko Nummi for not only being a valuable guest star in our pub quiz team but also providing a great example of a thesis and thesis defending.

I am very grateful for the grants that I received from the Orion Research Foundation for the data collection for study IV and forthcoming publications focusing on subjects with fatal PE. In addition I want to thank Helsingin Lääkäriyhdistys for grant that enabled the proofreading and layout of this thesis.

My family has also set the bar high in the academic world and now I will become the fourth PhD in the Sane family. I want to thank my brother Petri for using traditional motivational methods by undermining my educational status as a simple doctor and always offering support for IT problems when needed. In addition, I want to thank my brother Jussi for finding time to proofread my articles despite fighting simultaneously against the corona crisis. As a fairly recent parent I understand that none of this would have been possible without the love of my parents who have supported me at every turn of my life and not forgetting their help in always taking care of Ilona and Klaus when needed. The support given to our family from my mother-in-law, Tuulikki, has also been extremely important.

Lastly, I want to thank my wife Riikka, whose support and love have ensured that I was able to push this project along during the intensive years of residency. Life with you, the love of my life, has offered me the best distraction from this mostly frustrating life of part-time 'scientist' and no words can express how fortunate I am to be with you. Finally, Ilona and Klaus thank you for reminding me what is really important in life every morning at 6 a.m.

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14. Original publications